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(54) Title: NOVEL PURIFIED POLYPEPTIDES FROM PSEUDOMONAS AERUGINOSA

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(57) Abstract: The present invention relates to novel drug targets for pathogenic bacteria. Accordingly, the invention provides purified protein comprising the amino acid sequence set forth in SEQ ID NO:4. The invention also provides biochemical and biophysical characteristics of the polypeptides of the invention.

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***NOVEL PURIFIED POLYPEPTIDES FROM PSEUDOMONAS AERUGINOSA*****RELATED APPLICATION INFORMATION**

This application claims the benefit of priority to the following U.S. Provisional

5 Patent Applications, all of which applications are hereby incorporated by reference in their entirety.

<i>Provisional Application Number</i>	<i>Filing Date</i>
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**INTRODUCTION**

The discovery of novel antimicrobial agents that work by novel mechanisms is a  
10 problem researchers in all fields of drug development face today. The increasing prevalence of drug-resistant pathogens (bacteria, fungi, parasites, etc.) has led to significantly higher mortality rates from infectious diseases and currently presents a serious crisis worldwide. Despite the introduction of second and third generation antimicrobial drugs, certain pathogens have developed resistance to all currently available drugs.

15 One of the problems contributing to the development of multiple drug resistant pathogens is the limited number of protein targets for antimicrobial drugs. Many of the antibiotics currently in use are structurally related or act through common targets or pathways. Accordingly, adaptive mutation of a single gene may render a pathogenic species resistant to multiple classes of antimicrobial drugs. Therefore, the rapid discovery  
20 of drug targets is urgently needed in order to combat the constantly evolving threat by such infectious microorganisms.

Recent advances in bacterial and viral genomics research provides an opportunity for rapid progress in the identification of drug targets. The complete genomic sequences for a number of microorganisms are available. However, knowledge of the complete  
25 genomic sequence is only the first step in a long process toward discovery of a viable drug target. The genomic sequence must be annotated to identify open reading frames (ORFs), the essentiality of the protein encoded by the ORF must be determined and the mechanism of action of the gene product must be determined in order to develop a targeted approach to drug discovery.

30 There are a variety of computer programs available to annotate genomic sequences. Genome annotation involves both identification of genes as well assignment of function

thereto based on sequence comparison to homologous proteins with known or predicted functions. However, genome annotation has turned out to be much more of an art than a science. Factors such as splice variants and sequencing errors coupled with the particular algorithms and databases used to annotate the genome can result in significantly different 5 annotations for the same genome. For example, upon reanalysis of the genome of *Mycoplasma pneumoniae* using more rigorous sequence comparisons coupled with molecular biological techniques, such as gel electrophoresis and mass spectrometry, researchers were able to identify several previously unidentified coding sequences, to dismiss a previous identified coding sequence as a likely pseudogene, and to adjust the 10 length of several previously defined ORFs (Dandkar et al. (2000) Nucl. Acids Res. 28(17): 3278-3288). Furthermore, while overall conservation between amino acid sequences generally indicates a conservation of structure and function, specific changes at key residues can lead to significant variation in the biochemical and biophysical properties of a protein. In a comparison of three different functional annotations of the *Mycoplasma* 15 *genitalium* genome, it was discovered that some genes were assigned three different functions and it was estimated that the overall error rate in the annotations was at least 8% (Brenner (1999) Trends Genet 15(4): 132-3). Accordingly, molecular biological techniques are required to ensure proper genome annotation and identify valid drug targets.

However, confirmation of genome annotation using molecular biological techniques 20 is not an easy proposition due to the unpredictability in expression and purification of polypeptide sequences. Further, in order to carry out structural studies to validate proteins as potential drug targets, it is generally necessary to modify the native proteins in order to facilitate these analyses, e.g., by labeling the protein (e.g., with a heavy atom, isotopic label, polypeptide tag, etc.) or by creating fragments of the polypeptide corresponding to 25 functional domains of a multi-domain protein. Moreover, it is well-known that even small changes in the amino acid sequence of a protein may lead to dramatic affects on protein solubility (Eberstadt et al. (1998) Nature 392: 941-945). Accordingly, genome-wide validation of protein targets will require considerable effort even in light of the sequence of the entire genome of an organism and/or purification conditions for homologs of a 30 particular target.

We have developed reliable, high throughput methods to address some of the shortcomings identified above. In part, using these methods, we have now identified, expressed, and purified a novel antimicrobial target from *Pseudomonas aeruginosa*, or *P.*

*aeruginosa*. Various biophysical, bioinformatic and biochemical studies have been used to characterize the structure and function of the polypeptides of the invention.

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## 20 SUMMARY OF THE INVENTION

As part of an effort at genome-wide structural and functional characterization of microbial targets, the present invention provides polypeptides from *P. aeruginosa*. In various aspects, the invention provides the nucleic acid and amino acid sequences of the polypeptides of the invention. The invention also provides purified, soluble forms of the

25 polypeptides of the invention suitable for structural and functional characterization using a variety of techniques, including, for example, affinity chromatography, mass spectrometry, NMR and x-ray crystallography. The invention further provides modified versions of the polypeptides of the invention to facilitate characterization, including polypeptides labeled with isotopic or heavy atoms and fusion proteins.

30 A polypeptide of the invention has been crystallized and its structure solved as described in detail below, thereby providing information about the structure of the polypeptide, and druggable regions, domains and the like contained therein, all of which may be used in rational-based drug design efforts.

In general, the biological activity of a polypeptide of the invention is expected to be  
35 characterized as having a biochemical activity substantially similar to that of peptidyl-tRNA hydrolase, having the gene designation of *pth*, as described in more detail below.

This assignment has been confirmed by solving the X-ray structure of a polypeptide of the invention.

All of the information learned and described herein about the polypeptides of the invention may be used to design modulators of one or more of their biological activities. In 5 particular, information critical to the design of therapeutic and diagnostic molecules, including, for example, the protein domain, druggable regions, structural information, and the like for the polypeptides of the invention is now available or attainable as a result of the ability to prepare, purify and characterize them, and domains, fragments, variants and derivatives thereof.

10 In other aspects of the invention, structural and functional information about the polypeptides of the invention has and will be obtained. Such information, for example, may be incorporated into databases containing information on the polypeptides of the invention, as well as other polypeptide targets from other microbial species. Such databases will provide investigators with a powerful tool to analyze the polypeptides of the 15 invention and aid in the rapid discovery and design of therapeutic and diagnostic molecules.

In another aspect, modulators, inhibitors, agonists or antagonists against the polypeptides of the invention, or biological complexes containing them, or orthologues thereto, may be used to treat any disease or other treatable condition of a patient (including humans and animals), and particularly a disease caused by *P. aeruginosa*, such as, for 20 example, one of the following: osteomyelitis, otitis externa, conjunctivitis, keratitis, endophthalmitis, alveolar necrosis, vascular invasion, bacteremia, and burn infection.

The present invention further allows relationships between polypeptides from the same and multiple species to be compared by isolating and studying the various polypeptides of the invention and other proteins. By such comparison studies, which may 25 involve multi-variable analysis as appropriate, it is possible to identify drugs that will affect multiple species or drugs that will affect one or a few species. In such a manner, so-called "wide spectrum" and "narrow spectrum" anti-infectives may be identified. Alternatively, drugs that are selective for one or more bacterial or other non-mammalian species, and not for one or more mammalian species (especially human), may be identified (and vice-versa).

30 In other embodiments, the invention contemplates kits including the subject nucleic acids, polypeptides, crystallized polypeptides, antibodies, and other subject materials, and optionally instructions for their use. Uses for such kits include, for example, diagnostic and therapeutic applications.

The embodiments and practices of the present invention, other embodiments, and their features and characteristics, will be apparent from the description, figures and claims that follow, with all of the claims hereby being incorporated by this reference into this Summary.

5

#### BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 shows the nucleic acid coding sequence for an exemplary polypeptide of the invention as predicted from the genomic sequence of *P. aeruginosa* (SEQ ID NO: 1). This predicted nucleic acid coding sequence was cloned and sequenced to produce the 10 polynucleotide sequence shown in FIGURE 2 (SEQ ID NO: 3).

FIGURE 2 shows the amino acid sequence for an exemplary polypeptide of the invention as predicted from the nucleotide sequence shown in FIGURE 1 (SEQ ID NO: 2).

FIGURE 3 shows the experimentally determined nucleic acid coding sequence for an exemplary polypeptide of the invention (SEQ ID NO: 3).

15

FIGURE 4 shows the amino acid sequence for the exemplary polypeptide of the invention as predicted from the nucleotide sequence shown in FIGURE 3 (SEQ ID NO: 4).

FIGURE 5 shows the primer sequences used to amplify the nucleic acid of SEQ ID NO: 3. The primers are SEQ ID NO: 5 and SEQ ID NO: 6.

20

FIGURE 6 contains Table 1, which provides among other things a variety of data and other information on the polypeptides of the invention.

FIGURE 7 contains Table 2, which provides the results of several bioinformatic analyses relating to SEQ ID NO: 2.

FIGURE 8 contains Table 3, which shows information related to the x-ray structure for a polypeptide of the invention as described more fully in EXAMPLE 16.

25

FIGURE 9 lists the atomic structure coordinates for a polypeptide of the invention derived from x-ray diffraction from a crystal of such polypeptide, as described in more detail in EXAMPLE 16. There are multiple pages to FIGURE 9, labeled 1, 2, 3, etc. The information in such Figure is presented in the following tabular format, with a generic entry provided as an example:

Record Header	No.	Atom Type	Resi -due	Residue Number	X	Y	Z	OCC	B
ATOM 1	1	CB	HIS	1	4.497	15.607	34.172	1	70.54

In the table, "Record Header" describes the row type, such as "ATOM". "No." refers to the row number. The first "Atom Type" column refers to the atom whose coordinates are measured, with the first letter in the column identifying the atom by its elemental symbol and the subsequent letter defining the location of the atom in the amino acid residue or other molecule. "Residue" and "residue number" identifies the residue of the subject polypeptide. "X, Y, Z" crystallographically define the atomic position of the atom measured. "Occ" is an occupancy factor that refers to the fraction of the molecules in which each atom occupies the position specified by the coordinates. A value of "1" indicates that each atom has the same conformation, i.e., the same position, in all molecules of the crystal. "B" is a thermal factor that is related to the root mean square deviation in the position of the atom around the given atomic coordinate.

FIGURE 10 depicts a clustal V-based sequence alignment of the peptidyl-tRNA hydrolase protein sequences from six pathogens. The dark shading indicates conserved amino acids across species, with gray areas less conserved.

FIGURE 11 depicts a ribbon representation of a polypeptide of the invention, peptidyl-tRNA hydrolase from *P. aeruginosa*. The ribbon representation was generated with PYMOL as were all of the following figures (Delano, W.L., The PYMOL Molecular Graphics System (2002) DeLano Scientific, San Carlos, CA, USA). The helices and strands are colored in red and in green, respectively.

FIGURE 12 depicts the superimposition of the *E. coli* (colored in blue) and *P. aeruginosa* (colored in green) crystal structures for peptidyl-tRNA hydrolase.

FIGURE 13 depicts the location of the conserved residues in the three dimensional model of *P. aeruginosa* peptidyl-tRNA hydrolase in the druggable region that is believed to be a peptide-binding active site region, as described in more detail below. The strictly conserved residues among peptidyl-tRNA hydrolase polypeptides from various sources are colored in red. Conservative substitutions are colored in orange and variables residues are colored in yellow. (A) Ribbon diagram topped with side-chains drawn as red, orange and yellow sticks. (B) Molecular surface diagram of a monomer of *P. aeruginosa* peptidyl-tRNA hydrolase shown in roughly the same orientation.

FIGURE 14 depicts close views of what is believed to be the tRNA binding active site channel, as described in detail below. (A) *P. aeruginosa* peptidyl-tRNA hydrolase, with the most conserved residues area colored in red and the less conserved residues area in

blue, based on the data from FIGURE 10. (B) Cross-section of the active site channel. In *E. coli* peptidyl-tRNA hydrolase (on the right), a tryptophan residue partially occludes the mid-section of the channel, as described in greater detail below.

## 5 DETAILED DESCRIPTION OF THE INVENTION

### *1. Definitions*

For convenience, certain terms employed in the specification, examples, and appended claims are collected here. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

The term "amino acid" is intended to embrace all molecules, whether natural or synthetic, which include both an amino functionality and an acid functionality and capable of being included in a polymer of naturally-occurring amino acids. Exemplary amino acids include naturally-occurring amino acids; analogs, derivatives and congeners thereof; amino acid analogs having variant side chains; and all stereoisomers of any of any of the foregoing.

The term "binding" refers to an association, which may be a stable association, between two molecules, e.g., between a polypeptide of the invention and a binding partner, due to, for example, electrostatic, hydrophobic, ionic and/or hydrogen-bond interactions under physiological conditions.

A "comparison window," as used herein, refers to a conceptual segment of at least 20 contiguous amino acid positions wherein a protein sequence may be compared to a reference sequence of at least 20 contiguous amino acids and wherein the portion of the protein sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2: 482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48: 443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. (U.S.A.) 85:

2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by  
5 the various methods may be identified.

The term "complex" refers to an association between at least two moieties (e.g. chemical or biochemical) that have an affinity for one another. Examples of complexes include associations between antigen/antibodies, lectin/avidin, target polynucleotide/probe oligonucleotide, antibody/anti-antibody, receptor/ligand, enzyme/ligand, polypeptide/polypeptide, polypeptide/polynucleotide, polypeptide/co-factor, polypeptide/substrate, polypeptide/inhibitor, polypeptide/small molecule, and the like. "Member of a complex" refers to one moiety of the complex, such as an antigen or ligand. "Protein complex" or "polypeptide complex" refers to a complex comprising at least one polypeptide.  
10

The term "conserved residue" refers to an amino acid that is a member of a group of  
15 amino acids having certain common properties. The term "conservative amino acid substitution" refers to the substitution (conceptually or otherwise) of an amino acid from one such group with a different amino acid from the same group. A functional way to define common properties between individual amino acids is to analyze the normalized frequencies of amino acid changes between corresponding proteins of homologous  
20 organisms (Schulz, G. E. and R. H. Schirmer, Principles of Protein Structure, Springer-Verlag). According to such analyses, groups of amino acids may be defined where amino acids within a group exchange preferentially with each other, and therefore resemble each other most in their impact on the overall protein structure (Schulz, G. E. and R. H. Schirmer, Principles of Protein Structure, Springer-Verlag). One example of a set of amino  
25 acid groups defined in this manner include: (i) a charged group, consisting of Glu and Asp, Lys, Arg and His, (ii) a positively-charged group, consisting of Lys, Arg and His, (iii) a negatively-charged group, consisting of Glu and Asp, (iv) an aromatic group, consisting of Phe, Tyr and Trp, (v) a nitrogen ring group, consisting of His and Trp, (vi) a large aliphatic nonpolar group, consisting of Val, Leu and Ile, (vii) a slightly-polar group, consisting of  
30 Met and Cys, (viii) a small-residue group, consisting of Ser, Thr, Asp, Asn, Gly, Ala, Glu, Gln and Pro, (ix) an aliphatic group consisting of Val, Leu, Ile, Met and Cys, and (x) a small hydroxyl group consisting of Ser and Thr.

The term "domain", when used in connection with a polypeptide, refers to a specific region within such polypeptide that comprises a particular structure or mediates a particular function. In the typical case, a domain of a polypeptide of the invention is a fragment of the polypeptide. In certain instances, a domain is a structurally stable domain, as evidenced, 5 for example, by mass spectroscopy, or by the fact that a modulator may bind to a druggable region of the domain.

The term "druggable region", when used in reference to a polypeptide, nucleic acid, complex and the like, refers to a region of the molecule which is a target or is a likely target 10 for binding a modulator. For a polypeptide, a druggable region generally refers to a region wherein several amino acids of a polypeptide would be capable of interacting with a modulator or other molecule. For a polypeptide or complex thereof, exemplary druggable regions including binding pockets and sites, enzymatic active sites, interfaces between domains of a polypeptide or complex, surface grooves or contours or surfaces of a polypeptide or complex which are capable of participating in interactions with another 15 molecule. In certain instances, the interacting molecule is another polypeptide, which may be naturally-occurring. In other instances, the druggable region is on the surface of the molecule.

Druggable regions may be described and characterized in a number of ways. For 20 example, a druggable region may be characterized by some or all of the amino acids that make up the region, or the backbone atoms thereof, or the side chain atoms thereof (optionally with or without the C<sub>α</sub> atoms). Alternatively, in certain instances, the volume of a druggable region corresponds to that of a carbon based molecule of at least about 200 amu and often up to about 800 amu. In other instances, it will be appreciated that the volume of such region may correspond to a molecule of at least about 600 amu and often up 25 to about 1600 amu or more.

Alternatively, a druggable region may be characterized by comparison to other regions on the same or other molecules. For example, the term "affinity region" refers to a druggable region on a molecule (such as a polypeptide of the invention) that is present in 30 several other molecules, in so much as the structures of the same affinity regions are sufficiently the same so that they are expected to bind the same or related structural analogs. An example of an affinity region is an ATP-binding site of a protein kinase that is found in several protein kinases (whether or not of the same origin). The term "selectivity region" refers to a druggable region of a molecule that may not be found on other

molecules, in so much as the structures of different selectivity regions are sufficiently different so that they are not expected to bind the same or related structural analogs. An exemplary selectivity region is a catalytic domain of a protein kinase that exhibits specificity for one substrate. In certain instances, a single modulator may bind to the same 5 affinity region across a number of proteins that have a substantially similar biological function, whereas the same modulator may bind to only one selectivity region of one of those proteins.

Continuing with examples of different druggable regions, the term "undesired region" refers to a druggable region of a molecule that upon interacting with another 10 molecule results in an undesirable affect. For example, a binding site that oxidizes the interacting molecule (such as P-450 activity) and thereby results in increased toxicity for the oxidized molecule may be deemed a "undesired region". Other examples of potential undesired regions includes regions that upon interaction with a drug decrease the membrane permeability of the drug, increase the excretion of the drug, or increase the blood brain 15 transport of the drug. It may be the case that, in certain circumstances, an undesired region will no longer be deemed an undesired region because the affect of the region will be favorable, e.g., a drug intended to treat a brain condition would benefit from interacting with a region that resulted in increased blood brain transport, whereas the same region could be deemed undesirable for drugs that were not intended to be delivered to the brain.

When used in reference to a druggable region, the "selectivity" or "specificity" of a molecule such as a modulator to a druggable region may be used to describe the binding between the molecule and a druggable region. For example, the selectivity of a modulator with respect to a druggable region may be expressed by comparison to another modulator, using the respective values of  $K_d$  (i.e., the dissociation constants for each modulator-druggable region complex) or, in cases where a biological effect is observed below the  $K_d$ , the ratio of the respective EC<sub>50</sub>'s (i.e., the concentrations that produce 50% of the maximum response for the modulator interacting with each druggable region).

A "fusion protein" or "fusion polypeptide" refers to a chimeric protein as that term is known in the art and may be constructed using methods known in the art. In many 30 examples of fusion proteins, there are two different polypeptide sequences, and in certain cases, there may be more. The sequences may be linked in frame. A fusion protein may include a domain which is found (albeit in a different protein) in an organism which also expresses the first protein, or it may be an "interspecies", "intergenic", etc. fusion expressed

by different kinds of organisms. In various embodiments, the fusion polypeptide may comprise one or more amino acid sequences linked to a first polypeptide. In the case where more than one amino acid sequence is fused to a first polypeptide, the fusion sequences may be multiple copies of the same sequence, or alternatively, may be different amino acid  
5 sequences. The fusion polypeptides may be fused to the N-terminus, the C-terminus, or the N- and C-terminus of the first polypeptide. Exemplary fusion proteins include polypeptides comprising a glutathione S-transferase tag (GST-tag), histidine tag (His-tag), an immunoglobulin domain or an immunoglobulin binding domain.

The term "gene" refers to a nucleic acid comprising an open reading frame encoding  
10 a polypeptide having exon sequences and optionally intron sequences. The term "intron" refers to a DNA sequence present in a given gene which is not translated into protein and is generally found between exons.

The term "having substantially similar biological activity", when used in reference to two polypeptides, refers to a biological activity of a first polypeptide which is  
15 substantially similar to at least one of the biological activities of a second polypeptide. A substantially similar biological activity means that the polypeptides carry out a similar function, e.g., a similar enzymatic reaction or a similar physiological process, etc. For example, two homologous proteins may have a substantially similar biological activity if they are involved in a similar enzymatic reaction, e.g., they are both kinases which catalyze  
20 phosphorylation of a substrate polypeptide, however, they may phosphorylate different regions on the same protein substrate or different substrate proteins altogether. Alternatively, two homologous proteins may also have a substantially similar biological activity if they are both involved in a similar physiological process, e.g., transcription. For example, two proteins may be transcription factors, however, they may bind to different  
25 DNA sequences or bind to different polypeptide interactors. Substantially similar biological activities may also be associated with proteins carrying out a similar structural role, for example, two membrane proteins.

The term "isolated polypeptide" refers to a polypeptide, in certain embodiments prepared from recombinant DNA or RNA, or of synthetic origin, or some combination  
30 thereof, which (1) is not associated with proteins that it is normally found with in nature, (2) is isolated from the cell in which it normally occurs, (3) is isolated free of other proteins from the same cellular source, e.g. free of other *P. aeruginosa* proteins, (4) is expressed by a cell from a different species, or (5) does not occur in nature.

The term "isolated nucleic acid" refers to a polynucleotide of genomic, cDNA, or synthetic origin or some combination there of, which (1) is not associated with the cell in which the "isolated nucleic acid" is found in nature, or (2) is operably linked to a polynucleotide to which it is not linked in nature.

5       The terms "label" or "labeled" refer to incorporation or attachment, optionally covalently or non-covalently, of a detectable marker into a molecule, such as a polypeptide. Various methods of labeling polypeptides are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes, fluorescent labels, heavy atoms, enzymatic labels or reporter genes, chemiluminescent groups, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). Examples and use of such labels are described in more detail below. In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

10      The term "mammal" is known in the art, and exemplary mammals include humans, primates, bovines, porcines, canines, felines, and rodents (e.g., mice and rats).

15      The term "modulation", when used in reference to a functional property or biological activity or process (e.g., enzyme activity or receptor binding), refers to the capacity to either up regulate (e.g., activate or stimulate), down regulate (e.g., inhibit or suppress) or otherwise change a quality of such property, activity or process. In certain instances, such regulation may be contingent on the occurrence of a specific event, such as activation of a signal transduction pathway, and/or may be manifest only in particular cell types.

20      The term "modulator" refers to a polypeptide, nucleic acid, macromolecule, complex, molecule, small molecule, compound, species or the like (naturally-occurring or non-naturally-occurring), or an extract made from biological materials such as bacteria, plants, fungi, or animal cells or tissues, that may be capable of causing modulation. Modulators may be evaluated for potential activity as inhibitors or activators (directly or indirectly) of a functional property, biological activity or process, or combination of them,

25      (e.g., agonist, partial antagonist, partial agonist, inverse agonist, antagonist, anti-microbial agents, inhibitors of microbial infection or proliferation, and the like) by inclusion in assays. In such assays, many modulators may be screened at one time. The activity of a modulator may be known, unknown or partially known.

30

The term "motif" refers to an amino acid sequence that is commonly found in a protein of a particular structure or function. Typically, a consensus sequence is defined to represent a particular motif. The consensus sequence need not be strictly defined and may contain positions of variability, degeneracy, variability of length, etc. The consensus 5 sequence may be used to search a database to identify other proteins that may have a similar structure or function due to the presence of the motif in its amino acid sequence. For example, on-line databases may be searched with a consensus sequence in order to identify other proteins containing a particular motif. Various search algorithms and/or programs may be used, including FASTA, BLAST or ENTREZ. FASTA and BLAST are available 10 as a part of the GCG sequence analysis package (University of Wisconsin, Madison, Wis.). ENTREZ is available through the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD.

The term "naturally-occurring", as applied to an object, refers to the fact that an object may be found in nature. For example, a polypeptide or polynucleotide sequence that 15 is present in an organism (including bacteria) that may be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally-occurring.

The term "nucleic acid" refers to a polymeric form of nucleotides, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The 20 terms should also be understood to include, as equivalents, analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides.

The term "nucleic acid of the invention" refers to a nucleic acid encoding a polypeptide of the invention, e.g., a nucleic acid comprising a sequence consisting of, or 25 consisting essentially of, the polynucleotide sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 3. A nucleic acid of the invention may comprise all, or a portion of: the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 3; a nucleotide sequence at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 1 or SEQ ID NO: 3; a nucleotide sequence that hybridizes under stringent conditions to SEQ ID NO: 1 or SEQ ID 30 NO: 3; nucleotide sequences encoding polypeptides that are functionally equivalent to polypeptides of the invention; nucleotide sequences encoding polypeptides at least about 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% homologous or identical with an amino acid

sequence of SEQ ID NO: 2 or SEQ ID NO: 4; nucleotide sequences encoding polypeptides having an activity of a polypeptide of the invention and having at least about 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99% or more homology or identity with SEQ ID NO: 2 or SEQ ID NO: 4; nucleotide sequences that differ by 1 to about 2, 3, 5, 7, 10, 15, 20, 30, 50, 5 75 or more nucleotide substitutions, additions or deletions, such as allelic variants, of SEQ ID NO: 1 and SEQ ID NO: 3; nucleic acids derived from and evolutionarily related to SEQ ID NO: 1 or SEQ ID NO: 3; and complements of, and nucleotide sequences resulting from the degeneracy of the genetic code, for all of the foregoing and other nucleic acids of the invention. Nucleic acids of the invention also include homologs, e.g., orthologs and 10 paralogs, of SEQ ID NO: 1 or SEQ ID NO: 3 and also variants of SEQ ID NO: 1 or SEQ ID NO: 3 which have been codon optimized for expression in a particular organism (e.g., host cell).

The term "operably linked", when describing the relationship between two nucleic acid regions, refers to a juxtaposition wherein the regions are in a relationship permitting 15 them to function in their intended manner. For example, a control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences, such as when the appropriate molecules (e.g., inducers and polymerases) are bound to the control or regulatory sequence(s).

20 The term "phenotype" refers to the entire physical, biochemical, and physiological makeup of a cell, e.g., having any one trait or any group of traits.

The term "polypeptide", and the terms "protein" and "peptide" which are used interchangeably herein, refers to a polymer of amino acids. Exemplary polypeptides include gene products, naturally-occurring proteins, homologs, orthologs, paralogs, 25 fragments, and other equivalents, variants and analogs of the foregoing.

The terms "polypeptide fragment" or "fragment", when used in reference to a reference polypeptide, refers to a polypeptide in which amino acid residues are deleted as compared to the reference polypeptide itself, but where the remaining amino acid sequence is usually identical to the corresponding positions in the reference polypeptide. Such 30 deletions may occur at the amino-terminus or carboxy-terminus of the reference polypeptide, or alternatively both. Fragments typically are at least 5, 6, 8 or 10 amino acids long, at least 14 amino acids long, at least 20, 30, 40 or 50 amino acids long, at least 75 amino acids long, or at least 100, 150, 200, 300, 500 or more amino acids long. A fragment

can retain one or more of the biological activities of the reference polypeptide. In certain embodiments, a fragment may comprise a druggable region, and optionally additional amino acids on one or both sides of the druggable region, which additional amino acids may number from 5, 10, 15, 20, 30, 40, 50, or up to 100 or more residues. Further,  
5 fragments can include a sub-fragment of a specific region, which sub-fragment retains a function of the region from which it is derived. In another embodiment, a fragment may have immunogenic properties.

The term "polypeptide of the invention" refers to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, or an equivalent or  
10 fragment thereof, e.g., a polypeptide comprising a sequence consisting of, or consisting essentially of, the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4. Polypeptides of the invention include polypeptides comprising all or a portion of the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 with 1 to about 2, 3, 5, 7, 10, 15, 20, 30, 50, 75 or  
15 more conservative amino acid substitutions; an amino acid sequence that is at least 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 2 or SEQ ID NO: 4; and functional fragments thereof. Polypeptides of the invention also include homologs, e.g., orthologs and paralogs, of SEQ ID NO: 2 or SEQ ID NO: 4.

The term "purified" refers to an object species that is the predominant species  
20 present (i.e., on a molar basis it is more abundant than any other individual species in the composition). A "purified fraction" is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all species present. In making the determination of the purity of a species in solution or dispersion, the solvent or matrix in which the species is dissolved or dispersed is usually not included in such determination;  
25 instead, only the species (including the one of interest) dissolved or dispersed are taken into account. Generally, a purified composition will have one species that comprises more than about 80 percent of all species present in the composition, more than about 85%, 90%, 95%, 99% or more of all species present. The object species may be purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional  
30 detection methods) wherein the composition consists essentially of a single species. A skilled artisan may purify a polypeptide of the invention using standard techniques for protein purification in light of the teachings herein. Purity of a polypeptide may be

determined by a number of methods known to those of skill in the art, including for example, amino-terminal amino acid sequence analysis, gel electrophoresis, mass-spectrometry analysis and the methods described in the Exemplification section herein.

The terms "recombinant protein" or "recombinant polypeptide" refer to a 5 polypeptide which is produced by recombinant DNA techniques. An example of such techniques includes the case when DNA encoding the expressed protein is inserted into a suitable expression vector which is in turn used to transform a host cell to produce the protein or polypeptide encoded by the DNA.

A "reference sequence" is a defined sequence used as a basis for a sequence 10 comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length protein given in a sequence listing such as SEQ ID NO: 2 or SEQ ID NO: 4, or may comprise a complete protein sequence. Generally, a reference sequence is at least 200, 300 or 400 nucleotides in length, frequently at least 600 nucleotides in length, and often at least 800 nucleotides in length (or the protein equivalent if it is shorter 15 or longer in length). Because two proteins may each (1) comprise a sequence (i.e., a portion of the complete protein sequence) that is similar between the two proteins, and (2) may further comprise a sequence that is divergent between the two proteins, sequence comparisons between two (or more) proteins are typically performed by comparing sequences of the two proteins over a "comparison window" to identify and compare local 20 regions of sequence similarity.

The term "regulatory sequence" is a generic term used throughout the specification to refer to polynucleotide sequences, such as initiation signals, enhancers, regulators and promoters, that are necessary or desirable to affect the expression of coding and non-coding 25 sequences to which they are operably linked. Exemplary regulatory sequences are described in Goeddel; *Gene Expression Technology: Methods in Enzymology*, Academic Press, San Diego, CA (1990), and include, for example, the early and late promoters of SV40, adenovirus or cytomegalovirus immediate early promoter, the lac system, the trp system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda, the control regions 30 for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast  $\alpha$ -mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control

the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. The nature and use of such control sequences may differ depending upon the host organism. In prokaryotes, such regulatory sequences generally include promoter, ribosomal binding site, and transcription termination sequences. The term

5 "regulatory sequence" is intended to include, at a minimum, components whose presence may influence expression, and may also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. In certain embodiments, transcription of a polynucleotide sequence is under the control of a promoter sequence (or other regulatory sequence) which controls the expression of the

10 polynucleotide in a cell-type in which expression is intended. It will also be understood that the polynucleotide can be under the control of regulatory sequences which are the same or different from those sequences which control expression of the naturally-occurring form of the polynucleotide.

The term "reporter gene" refers to a nucleic acid comprising a nucleotide sequence,

15 encoding a protein that is readily detectable either by its presence or activity, including, but not limited to, luciferase, fluorescent protein (e.g., green fluorescent protein), chloramphenicol acetyl transferase,  $\beta$ -galactosidase, secreted placental alkaline phosphatase,  $\beta$ -lactamase, human growth hormone, and other secreted enzyme reporters. Generally, a reporter gene encodes a polypeptide not otherwise produced by the host cell,

20 which is detectable by analysis of the cell(s), e.g., by the direct fluorometric, radioisotopic or spectrophotometric analysis of the cell(s) and preferably without the need to kill the cells for signal analysis. In certain instances, a reporter gene encodes an enzyme, which produces a change in fluorometric properties of the host cell, which is detectable by qualitative, quantitative or semiquantitative function or transcriptional activation.

25 Exemplary enzymes include esterases,  $\beta$ -lactamase, phosphatases, peroxidases, proteases (tissue plasminogen activator or urokinase) and other enzymes whose function may be detected by appropriate chromogenic or fluorogenic substrates known to those skilled in the art or developed in the future.

The term "sequence homology" refers to the proportion of base matches between

30 two nucleic acid sequences or the proportion of amino acid matches between two amino acid sequences. When sequence homology is expressed as a percentage, e.g., 50%, the percentage denotes the proportion of matches over the length of sequence from a desired

sequence (e.g., SEQ. ID NO: 1) that is compared to some other sequence. Gaps (in either of the two sequences) are permitted to maximize matching; gap lengths of 15 bases or less are usually used, 6 bases or less are used more frequently, with 2 bases or less used even more frequently. The term "sequence identity" means that sequences are identical (i.e., on 5 a nucleotide-by-nucleotide basis for nucleic acids or amino acid-by-amino acid basis for polypeptides) over a window of comparison. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the comparison window, determining the number of positions at which the identical amino acids occurs in both sequences to yield the number of matched positions, dividing the number of matched 10 positions by the total number of positions in the comparison window, and multiplying the result by 100 to yield the percentage of sequence identity. Methods to calculate sequence identity are known to those of skill in the art and described in further detail below.

The term "small molecule" refers to a compound, which has a molecular weight of less than about 5 kD, less than about 2.5 kD, less than about 1.5 kD, or less than about 0.9 15 kD. Small molecules may be, for example, nucleic acids, peptides, polypeptides, peptide nucleic acids, peptidomimetics, carbohydrates, lipids or other organic (carbon containing) or inorganic molecules. Many pharmaceutical companies have extensive libraries of chemical and/or biological mixtures, often fungal, bacterial, or algal extracts, which can be screened with any of the assays of the invention. The term "small organic molecule" refers 20 to a small molecule that is often identified as being an organic or medicinal compound, and does not include molecules that are exclusively nucleic acids, peptides or polypeptides.

The term "soluble" as used herein with reference to a polypeptide of the invention or other protein, means that upon expression in cell culture, at least some portion of the polypeptide or protein expressed remains in the cytoplasmic fraction of the cell and does 25 not fractionate with the cellular debris upon lysis and centrifugation of the lysate. Solubility of a polypeptide may be increased by a variety of art recognized methods, including fusion to a heterologous amino acid sequence, deletion of amino acid residues, amino acid substitution (e.g., enriching the sequence with amino acid residues having hydrophilic side chains), and chemical modification (e.g., addition of hydrophilic groups). 30 The solubility of polypeptides may be measured using a variety of art recognized techniques, including, dynamic light scattering to determine aggregation state, UV absorption, centrifugation to separate aggregated from non-aggregated material, and SDS gel electrophoresis (e.g., the amount of protein in the soluble fraction is compared to the

amount of protein in the soluble and insoluble fractions combined). When expressed in a host cell, the polypeptides of the invention may be at least about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more soluble, e.g., at least about 1%, 2%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more of the total amount of protein

5 expressed in the cell is found in the cytoplasmic fraction. In certain embodiments, a one liter culture of cells expressing a polypeptide of the invention will produce at least about 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 30, 40, 50 milligrams or more of soluble protein. In an exemplary embodiment, a polypeptide of the invention is at least about 10% soluble and will produce at least about 1 milligram of protein from a one liter cell culture.

10 The term "specifically hybridizes" refers to detectable and specific nucleic acid binding. Polynucleotides, oligonucleotides and nucleic acids of the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. Stringent conditions may be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and nucleic acids of the invention and a nucleic acid sequence of interest will be at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 98%, 99%, or more. In certain instances, hybridization and washing conditions are performed under stringent conditions according to conventional hybridization procedures and as described further herein.

15 The terms "stringent conditions" or "stringent hybridization conditions" refer to conditions which promote specific hybridization between two complementary polynucleotide strands so as to form a duplex. Stringent conditions may be selected to be about 5°C lower than the thermal melting point ( $T_m$ ) for a given polynucleotide duplex at a defined ionic strength and pH. The length of the complementary polynucleotide strands and their GC content will determine the  $T_m$  of the duplex, and thus the hybridization conditions necessary for obtaining a desired specificity of hybridization. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the a polynucleotide sequence hybridizes to a perfectly matched complementary strand. In certain cases it may be desirable to increase the stringency of the hybridization conditions to be about equal to the  $T_m$  for a particular duplex.

20 25 30 A variety of techniques for estimating the  $T_m$  are available. Typically, G-C base pairs in a duplex are estimated to contribute about 3°C to the  $T_m$ , while A-T base pairs are

estimated to contribute about 2°C, up to a theoretical maximum of about 80-100°C. However, more sophisticated models of Tm are available in which G-C stacking interactions, solvent effects, the desired assay temperature and the like are taken into account. For example, probes can be designed to have a dissociation temperature (Td) of

5 approximately 60°C, using the formula:  $Td = (((((3 \times \#GC) + (2 \times \#AT)) \times 37) - 562) / \#bp) - 5$ ; where #GC, #AT, and #bp are the number of guanine-cytosine base pairs, the number of adenine-thymine base pairs, and the number of total base pairs, respectively, involved in the formation of the duplex.

Hybridization may be carried out in 5xSSC, 4xSSC, 3xSSC, 2xSSC, 1xSSC or 10 0.2xSSC for at least about 1 hour, 2 hours, 5 hours, 12 hours, or 24 hours. The temperature of the hybridization may be increased to adjust the stringency of the reaction, for example, from about 25°C (room temperature), to about 45°C, 50°C, 55°C, 60°C, or 65°C. The hybridization reaction may also include another agent affecting the stringency, for example, hybridization conducted in the presence of 50% formamide increases the stringency of 15 hybridization at a defined temperature.

The hybridization reaction may be followed by a single wash step, or two or more wash steps, which may be at the same or a different salinity and temperature. For example, the temperature of the wash may be increased to adjust the stringency from about 25°C (room temperature), to about 45°C, 50°C, 55°C, 60°C, 65°C, or higher. The wash step may 20 be conducted in the presence of a detergent, e.g., 0.1 or 0.2% SDS. For example, hybridization may be followed by two wash steps at 65°C each for about 20 minutes in 2xSSC, 0.1% SDS, and optionally two additional wash steps at 65°C each for about 20 minutes in 0.2xSSC, 0.1%SDS.

Exemplary stringent hybridization conditions include overnight hybridization at 25 65°C in a solution comprising, or consisting of, 50% formamide, 10xDenhardt (0.2% Ficoll, 0.2% Polyvinylpyrrolidone, 0.2% bovine serum albumin) and 200 g/ml of denatured carrier DNA, e.g., sheared salmon sperm DNA, followed by two wash steps at 65°C each for about 20 minutes in 2xSSC, 0.1% SDS, and two wash steps at 65°C each for about 20 minutes in 0.2xSSC, 0.1%SDS.

Hybridization may consist of hybridizing two nucleic acids in solution, or a nucleic acid in solution to a nucleic acid attached to a solid support, e.g., a filter. When one nucleic acid is on a solid support, a prehybridization step may be conducted prior to hybridization. Prehybridization may be carried out for at least about 1 hour, 3 hours or 10 hours in the 30

same solution and at the same temperature as the hybridization solution (without the complementary polynucleotide strand).

Appropriate stringency conditions are known to those skilled in the art or may be determined experimentally by the skilled artisan. See, for example, Current Protocols in

5 Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-12.3.6; Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; S. Agrawal (ed.) Methods in Molecular Biology, volume 20; Tijssen (1993) Laboratory Techniques in biochemistry and molecular biology-hybridization with nucleic acid probes, e.g., part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe  
10 assays", Elsevier, New York; and Tibanyenda, N. et al., Eur. J. Biochem. 139:19 (1984) and Ebel, S. et al., Biochem. 31:12083 (1992).

As applied to proteins, the term "substantial identity" means that two protein sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, typically share at least about 70 percent sequence identity, alternatively  
15 at least about 80, 85, 90, 95 percent sequence identity or more. In certain instances, residue positions that are not identical differ by conservative amino acid substitutions, which are described above.

The term "structural motif", when used in reference to a polypeptide, refers to a polypeptide that, although it may have different amino acid sequences, may result in a  
20 similar structure, wherein by structure is meant that the motif forms generally the same tertiary structure, or that certain amino acid residues within the motif, or alternatively their backbone or side chains (which may or may not include the C atoms of the side chains) are positioned in a like relationship with respect to one another in the motif.

The term "test compound" refers to a molecule to be tested by one or more  
25 screening method(s) as a putative modulator of a polypeptide of the invention or other biological entity or process. A test compound is usually not known to bind to a target of interest. The term "control test compound" refers to a compound known to bind to the target (e.g., a known agonist, antagonist, partial agonist or inverse agonist). The term "test compound" does not include a chemical added as a control condition that alters the function  
30 of the target to determine signal specificity in an assay. Such control chemicals or conditions include chemicals that 1) nonspecifically or substantially disrupt protein structure (e.g., denaturing agents (e.g., urea or guanidinium), chaotropic agents, sulphydryl reagents (e.g., dithiothreitol and  $\beta$ -mercaptoethanol), and proteases), 2) generally inhibit

cell metabolism (e.g., mitochondrial uncouplers) and 3) non-specifically disrupt electrostatic or hydrophobic interactions of a protein (e.g., high salt concentrations, or detergents at concentrations sufficient to non-specifically disrupt hydrophobic interactions). Further, the term "test compound" also does not include compounds known to be unsuitable

5 for a therapeutic use for a particular indication due to toxicity of the subject. In certain embodiments, various predetermined concentrations of test compounds are used for screening such as 0.01  $\mu$ M, 0.1  $\mu$ M, 1.0  $\mu$ M, and 10.0  $\mu$ M. Examples of test compounds include, but are not limited to, peptides, nucleic acids, carbohydrates, and small molecules.

10 The term "novel test compound" refers to a test compound that is not in existence as of the filing date of this application. In certain assays using novel test compounds, the novel test compounds comprise at least about 50%, 75%, 85%, 90%, 95% or more of the test compounds used in the assay or in any particular trial of the assay.

The term "therapeutically effective amount" refers to that amount of a modulator, drug or other molecule which is sufficient to effect treatment when administered to a subject in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

15 The term "transfection" means the introduction of a nucleic acid, e.g., an expression vector, into a recipient cell, which in certain instances involves nucleic acid-mediated gene transfer. The term "transformation" refers to a process in which a cell's genotype is changed as a result of the cellular uptake of exogenous nucleic acid. For example, a transformed cell may express a recombinant form of a polypeptide of the invention or antisense expression may occur from the transferred gene so that the expression of a naturally-occurring form of the gene is disrupted.

20 The term "transgene" means a nucleic acid sequence, which is partly or entirely heterologous to a transgenic animal or cell into which it is introduced, or, is homologous to an endogenous gene of the transgenic animal or cell into which it is introduced, but which is designed to be inserted, or is inserted, into the animal's genome in such a way as to alter 25 the genome of the cell into which it is inserted (e.g., it is inserted at a location which differs from that of the natural gene or its insertion results in a knockout). A transgene may include one or more regulatory sequences and any other nucleic acids, such as introns, that may be necessary for optimal expression.

The term "transgenic animal" refers to any animal, for example, a mouse, rat or other non-human mammal, a bird or an amphibian, in which one or more of the cells of the animal contain heterologous nucleic acid introduced by way of human intervention, such as by transgenic techniques well known in the art. The nucleic acid is introduced into the cell,

5 directly or indirectly, by way of deliberate genetic manipulation, such as by microinjection or by infection with a recombinant virus. The term genetic manipulation does not include classical cross-breeding, or *in vitro* fertilization, but rather is directed to the introduction of a recombinant DNA molecule. This molecule may be integrated within a chromosome, or it may be extrachromosomally replicating DNA. In the typical transgenic animals  
10 described herein, the transgene causes cells to express a recombinant form of a protein. However, transgenic animals in which the recombinant gene is silent are also contemplated.

The term "vector" refers to a nucleic acid capable of transporting another nucleic acid to which it has been linked. One type of vector which may be used in accord with the invention is an episome, i.e., a nucleic acid capable of extra-chromosomal replication.

15 Other vectors include those capable of autonomous replication and expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids" which refer to circular double stranded DNA molecules which, in their vector  
20 form are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which become known in the art subsequently hereto.

Unless otherwise indicated, all numbers expressing quantities of ingredients,  
25 reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in this specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention.

30

## *2. Polypeptides of the Invention*

The present invention makes available in a variety of embodiments soluble, purified and/or isolated forms of the polypeptides of the invention. Milligram quantities of an

exemplary polypeptide of the invention, SEQ ID NO: 4 (optionally with a tag, and optionally labeled), have been isolated in a highly purified form. The present invention provides for expressing and purifying polypeptides of the invention in quantities that equal or exceed the quantity of polypeptide(s) of the invention expressed and purified as provided  
5 in the Exemplification section below (or smaller amount(s) thereof, such as 25%, 33%, 50% or 75% of the amount(s) so expressed and/or purified).

In one aspect, the present invention contemplates an isolated polypeptide comprising (a) the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, (b) the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 with 1 to about 20  
10 conservative amino acid substitutions, deletions or additions, (c) an amino acid sequence that is at least 90% identical to SEQ ID NO: 2 or SEQ ID NO: 4 or (d) a functional fragment of a polypeptide having an amino acid sequence set forth in (a), (b) or (c). In another aspect, the present invention contemplates a composition comprising such an isolated polypeptide and less than about 10%, or alternatively 5%, or alternatively 1%,  
15 contaminating biological macromolecules or polypeptides.

It may be the case that the amino acid sequence of SEQ ID NO: 4 differs from that of SEQ ID NO: 2 by one or more amino acids. SEQ ID NO: 4 is determined from the experimentally determined nucleic acid sequence SED ID NO: 3, and SEQ ID NO: 2 is determined from SEQ ID NO: 1, which is obtained as described in EXAMPLE 1. In such a  
20 case, the present invention contemplates the specific amino acid sequences of SEQ ID NO: 2 and SEQ ID NO: 4, and variants thereof, as well as any differences (if any) in the polypeptides of the invention based on those SEQ ID NOS and nucleic acid sequences encoding the same.

In certain embodiments, a polypeptide of the invention is a fusion protein containing  
25 a domain which increases its solubility and/or facilitates its purification, identification, detection, and/or structural characterization. Exemplary domains, include, for example, glutathione S-transferase (GST), protein A, protein G, calmodulin-binding peptide, thioredoxin, maltose binding protein, HA, myc, poly arginine, poly His, poly His-Asp or FLAG fusion proteins and tags. Additional exemplary domains include domains that alter  
30 protein localization *in vivo*, such as signal peptides, type III secretion system-targeting peptides, transcytosis domains, nuclear localization signals, etc. In various embodiments, a polypeptide of the invention may comprise one or more heterologous fusions. Polypeptides may contain multiple copies of the same fusion domain or may contain fusions to two or

more different domains. The fusions may occur at the N-terminus of the polypeptide, at the C-terminus of the polypeptide, or at both the N- and C-terminus of the polypeptide. It is also within the scope of the invention to include linker sequences between a polypeptide of the invention and the fusion domain in order to facilitate construction of the fusion protein  
5 or to optimize protein expression or structural constraints of the fusion protein. In another embodiment, the polypeptide may be constructed so as to contain protease cleavage sites between the fusion polypeptide and polypeptide of the invention in order to remove the tag after protein expression or thereafter. Examples of suitable endoproteases, include, for example, Factor Xa and TEV proteases.

10 In another embodiment, a polypeptide of the invention may be modified so that its rate of traversing the cellular membrane is increased. For example, the polypeptide may be fused to a second peptide which promotes "transcytosis," e.g., uptake of the peptide by cells. The peptide may be a portion of the HIV transactivator (TAT) protein, such as the fragment corresponding to residues 37-62 or 48-60 of TAT, portions which have been  
15 observed to be rapidly taken up by a cell *in vitro* (Green and Loewenstein, (1989) Cell : 55:1179-1188). Alternatively, the internalizing peptide may be derived from the *Drosophila antennapedia* protein, or homologs thereof. The 60 amino acid long homeodomain of the homeo-protein antennapedia has been demonstrated to translocate through biological membranes and can facilitate the translocation of heterologous  
20 polypeptides to which it is coupled. Thus, polypeptides may be fused to a peptide consisting of about amino acids 42-58 of *Drosophila antennapedia* or shorter fragments for transcytosis (Derossi et al. (1996) J Biol Chem 271:18188-18193; Derossi et al. (1994) J Biol Chem 269:10444-10450; and Perez et al. (1992) J Cell Sci 102:717-722). The transcytosis polypeptide may also be a non-naturally-occurring membrane-translocating  
25 sequence (MTS), such as the peptide sequences disclosed in U.S. Patent No. 6,248,558.

In another embodiment, a polypeptide of the invention is labeled with an isotopic label to facilitate its detection and or structural characterization using nuclear magnetic resonance or another applicable technique. Exemplary isotopic labels include radioisotopic labels such as, for example, potassium-40 (<sup>40</sup>K), carbon-14 (<sup>14</sup>C), tritium (<sup>3</sup>H), sulphur-35  
30 (<sup>35</sup>S), phosphorus-32 (<sup>32</sup>P), technetium-99m (<sup>99m</sup>Tc), thallium-201 (<sup>201</sup>Tl), gallium-67 (<sup>67</sup>Ga), indium-111 (<sup>111</sup>In), iodine-123 (<sup>123</sup>I), iodine-131 (<sup>131</sup>I), yttrium-90 (<sup>90</sup>Y), samarium-153 (<sup>153</sup>Sm), rhodium-186 (<sup>186</sup>Rh), rhenium-188 (<sup>188</sup>Re), dysprosium-165 (<sup>165</sup>Dy) and holmium-166 (<sup>166</sup>Ho). The isotopic label may also be an atom with non zero nuclear spin,

including, for example, hydrogen-1 (<sup>1</sup>H), hydrogen-2 (<sup>2</sup>H), hydrogen-3 (<sup>3</sup>H), phosphorous-31 (<sup>31</sup>P), sodium-23 (<sup>23</sup>Na), nitrogen-14 (<sup>14</sup>N), nitrogen-15 (<sup>15</sup>N), carbon-13 (<sup>13</sup>C) and fluorine-19 (<sup>19</sup>F). In certain embodiments, the polypeptide is uniformly labeled with an isotopic label, for example, wherein at least 50%, 70%, 80%, 90%, 95%, or 98% of the

5 possible labels in the polypeptide are labeled, e.g., wherein at least 50%, 70%, 80%, 90%, 95%, or 98% of the nitrogen atoms in the polypeptide are <sup>15</sup>N, and/or wherein at least 50%, 70%, 80%, 90%, 95%, or 98% of the carbon atoms in the polypeptide are <sup>13</sup>C, and/or wherein at least 50%, 70%, 80%, 90%, 95%, or 98% of the hydrogen atoms in the polypeptide are <sup>2</sup>H. In other embodiments, the isotopic label is located in one or more specific locations within the polypeptide, for example, the label may be specifically incorporated into one or more of the leucine residues of the polypeptide. The invention also encompasses the embodiment wherein a single polypeptide comprises two, three or more different isotopic labels, for example, the polypeptide comprises both <sup>15</sup>N and <sup>13</sup>C labeling.

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In yet another embodiment, the polypeptides of the invention are labeled to facilitate

15 structural characterization using x-ray crystallography or another applicable technique. Exemplary labels include heavy atom labels such as, for example, cobalt, selenium, krypton, bromine, strontium, molybdenum, ruthenium, rhodium, palladium, silver, cadmium, tin, iodine, xenon, barium, lanthanum, cerium, praseodymium, neodymium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, lutetium, tantalum, tungsten, rhenium, osmium, iridium, platinum, gold, mercury, thallium, lead, thorium and uranium. In an exemplary embodiment, the polypeptide is labeled with seleno-methionine.

20

A variety of methods are available for preparing a polypeptide with a label, such as a radioisotopic label or heavy atom label. For example, in one such method, an expression 25 vector comprising a nucleic acid encoding a polypeptide is introduced into a host cell, and the host cell is cultured in a cell culture medium in the presence of a source of the label, thereby generating a labeled polypeptide. As indicated above, the extent to which a polypeptide may be labeled may vary.

In still another embodiment, the polypeptides of the invention are labeled with a 30 fluorescent label to facilitate their detection, purification, or structural characterization. In an exemplary embodiment, a polypeptide of the invention is fused to a heterologous polypeptide sequence which produces a detectable fluorescent signal, including, for example, green fluorescent protein (GFP), enhanced green fluorescent protein (EGFP),

*Renilla Reniformis* green fluorescent protein, GFPmut2, GFPuv4, enhanced yellow fluorescent protein (EYFP), enhanced cyan fluorescent protein (ECFP), enhanced blue fluorescent protein (EBFP), citrine and red fluorescent protein from discosoma (dsRED).

In other embodiments, the invention provides for polypeptides of the invention immobilized onto a solid surface, including, microtiter plates, slides, beads, films, etc. The polypeptides of the invention may be immobilized onto a "chip" as part of an array. An array, having a plurality of addresses, may comprise one or more polypeptides of the invention in one or more of those addresses. In one embodiment, the chip comprises one or more polypeptides of the invention as part of an array of *P. aeruginosa* polypeptide sequences.

In other embodiments, the invention provides for polypeptides of the invention immobilized onto a solid surface, including, plates, microtiter plates, slides, beads, particles, spheres, films, strands, precipitates, gels, sheets, tubing, containers, capillaries, pads, slices, etc. The polypeptides of the invention may be immobilized onto a "chip" as part of an array. An array, having a plurality of addresses, may comprise one or more polypeptides of the invention in one or more of those addresses. In one embodiment, the chip comprises one or more polypeptides of the invention as part of an array that contains at least some polypeptide sequences from *P. aeruginosa*.

In still other embodiments, the invention comprises the polypeptide sequences of the invention in computer readable format. The invention also encompasses a database comprising the polypeptide sequences of the invention.

In other embodiments, the invention relates to the polypeptides of the invention contained within a vessels useful for manipulation of the polypeptide sample. For example, the polypeptides of the invention may be contained within a microtiter plate to facilitate detection, screening or purification of the polypeptide. The polypeptides may also be contained within a syringe as a container suitable for administering the polypeptide to a subject in order to generate antibodies or as part of a vaccination regimen. The polypeptides may also be contained within an NMR tube in order to enable characterization by nuclear magnetic resonance techniques.

In still other embodiments, the invention relates to a crystallized polypeptide of the invention and crystallized polypeptides which have been mounted for examination by x-ray crystallography as described further below. In certain instances, a polypeptide of the invention in crystal form may be single crystals of various dimensions (e.g., micro-crystals)

or may be an aggregate of crystalline material. In another aspect, the present invention contemplates a crystallized complex including a polypeptide of the invention and one or more of the following: a co-factor (such as a salt, metal, nucleotide, oligonucleotide or polypeptide), a modulator, or a small molecule. In another aspect, the present invention  
5 contemplates a crystallized complex including a polypeptide of the invention and any other molecule or atom (such as a metal ion) that associates with the polypeptide *in vivo*.

In certain embodiments, polypeptides of the invention may be synthesized chemically, ribosomally in a cell free system, or ribosomally within a cell. Chemical synthesis of polypeptides of the invention may be carried out using a variety of art  
10 recognized methods, including stepwise solid phase synthesis, semi-synthesis through the conformationally-assisted re-ligation of peptide fragments, enzymatic ligation of cloned or synthetic peptide segments, and chemical ligation. Native chemical ligation employs a chemoselective reaction of two unprotected peptide segments to produce a transient thioester-linked intermediate.

The transient thioester-linked intermediate then  
15 spontaneously undergoes a rearrangement to provide the full length ligation product having a native peptide bond at the ligation site. Full length ligation products are chemically identical to proteins produced by cell free synthesis. Full length ligation products may be refolded and/or oxidized, as allowed, to form native disulfide-containing protein molecules.  
(see e.g., U.S. Patent Nos. 6,184,344 and 6,174,530; and T. W. Muir et al., *Curr. Opin. Biotech.* (1993): vol. 4, p 420; M. Miller, et al., *Science* (1989): vol. 246, p 1149; A. Wlodawer, et al., *Science* (1989): vol. 245, p 616; L. H. Huang, et al., *Biochemistry* (1991):  
vol. 30, p 7402; M. Schnolzer, et al., *Int. J. Pept. Prot. Res.* (1992): vol. 40, p 180-193; K. Rajarathnam, et al., *Science* (1994): vol. 264, p 90; R. E. Offord, "Chemical Approaches to Protein Engineering", in *Protein Design and the Development of New therapeutics and*

25 *Vaccines*, J. B. Hook, G. Poste, Eds., (Plenum Press, New York, 1990) pp. 253-282; C. J. A. Wallace, et al., *J. Biol. Chem.* (1992): vol. 267, p 3852; L. Abrahmsen, et al., *Biochemistry* (1991): vol. 30, p 4151; T. K. Chang, et al., *Proc. Natl. Acad. Sci. USA* (1994) 91: 12544-12548; M. Schnlzer, et al., *Science* (1992): vol., 3256, p 221; and K. Akaji, et al., *Chem. Pharm. Bull. (Tokyo)* (1985) 33: 184).

30 In certain embodiments, it may be advantageous to provide naturally-occurring or experimentally-derived homologs of a polypeptide of the invention. Such homologs may function in a limited capacity as a modulator to promote or inhibit a subset of the biological activities of the naturally-occurring form of the polypeptide. Thus, specific biological

effects may be elicited by treatment with a homolog of limited function, and with fewer side effects relative to treatment with agonists or antagonists which are directed to all of the biological activities of a polypeptide of the invention. For instance, antagonistic homologs may be generated which interfere with the ability of the wild-type polypeptide of the 5 invention to associate with certain proteins, but which do not substantially interfere with the formation of complexes between the native polypeptide and other cellular proteins.

Another aspect of the invention relates to polypeptides derived from the full-length polypeptides of the invention. Isolated peptidyl portions of those polypeptides may be obtained by screening polypeptides recombinantly produced from the corresponding 10 fragment of the nucleic acid encoding such polypeptides. In addition, fragments may be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. For example, proteins may be arbitrarily divided into fragments of desired length with no overlap of the fragments, or may be divided into overlapping fragments of a desired length. The fragments may be produced (recombinantly 15 or by chemical synthesis) and tested to identify those peptidyl fragments having a desired property, for example, the capability of functioning as a modulator of the polypeptides of the invention. In an illustrative embodiment, peptidyl portions of a protein of the invention may be tested for binding activity, as well as inhibitory ability, by expression as, for example, thioredoxin fusion proteins, each of which contains a discrete fragment of a 20 protein of the invention (see, for example, U.S. Patents 5,270,181 and 5,292,646; and PCT publication WO94/ 02502).

In another embodiment, truncated polypeptides may be prepared. Truncated polypeptides have from 1 to 20 or more amino acid residues removed from either or both the N- and C-termini. Such truncated polypeptides may prove more amenable to 25 expression, purification or characterization than the full-length polypeptide. For example, truncated polypeptides may prove more amenable than the full-length polypeptide to crystallization, to yielding high quality diffracting crystals or to yielding an HSQC spectrum with high intensity peaks and minimally overlapping peaks. In addition, the use of truncated polypeptides may also identify stable and active domains of the full-length 30 polypeptide that may be more amenable to characterization.

It is also possible to modify the structure of the polypeptides of the invention for such purposes as enhancing therapeutic or prophylactic efficacy, or stability (e.g., *ex vivo* shelf life, resistance to proteolytic degradation *in vivo*, etc.). Such modified polypeptides,

when designed to retain at least one activity of the naturally-occurring form of the protein, are considered "functional equivalents" of the polypeptides described in more detail herein. Such modified polypeptides may be produced, for instance, by amino acid substitution, deletion, or addition, which substitutions may consist in whole or part by conservative  
5 amino acid substitutions.

For instance, it is reasonable to expect that an isolated conservative amino acid substitution, such as replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, will not have a major affect on the biological activity of the resulting molecule. Whether a change in the amino acid sequence of a polypeptide  
10 results in a functional homolog may be readily determined by assessing the ability of the variant polypeptide to produce a response similar to that of the wild-type protein. Polypeptides in which more than one replacement has taken place may readily be tested in the same manner.

This invention further contemplates a method of generating sets of combinatorial  
15 mutants of polypeptides of the invention, as well as truncation mutants, and is especially useful for identifying potential variant sequences (e.g. homologs). The purpose of screening such combinatorial libraries is to generate, for example, homologs which may modulate the activity of a polypeptide of the invention, or alternatively, which possess novel activities altogether. Combinatorially-derived homologs may be generated which  
20 have a selective potency relative to a naturally-occurring protein. Such homologs may be used in the development of therapeutics.

Likewise, mutagenesis may give rise to homologs which have intracellular half-lives dramatically different than the corresponding wild-type protein. For example, the altered protein may be rendered either more stable or less stable to proteolytic degradation  
25 or other cellular process which result in destruction of, or otherwise inactivation of the protein. Such homologs, and the genes which encode them, may be utilized to alter protein expression by modulating the half-life of the protein. As above, such proteins may be used for the development of therapeutics or treatment.

In similar fashion, protein homologs may be generated by the present combinatorial  
30 approach to act as antagonists, in that they are able to interfere with the activity of the corresponding wild-type protein.

In a representative embodiment of this method, the amino acid sequences for a population of protein homologs are aligned, preferably to promote the highest homology

possible. Such a population of variants may include, for example, homologs from one or more species, or homologs from the same species but which differ due to mutation. Amino acids which appear at each position of the aligned sequences are selected to create a degenerate set of combinatorial sequences. In certain embodiments, the combinatorial library is produced by way of a degenerate library of genes encoding a library of polypeptides which each include at least a portion of potential protein sequences. For instance, a mixture of synthetic oligonucleotides may be enzymatically ligated into gene sequences such that the degenerate set of potential nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g. for phage display).

There are many ways by which the library of potential homologs may be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence may be carried out in an automatic DNA synthesizer, and the synthetic genes may then be ligated into an appropriate vector for expression. One purpose of a degenerate set of genes is to provide, in one mixture, all of the sequences encoding the desired set of potential protein sequences. The synthesis of degenerate oligonucleotides is well known in the art (see for example, Narang, SA (1983) *Tetrahedron* 39:3; Itakura et al., (1981) *Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules*, ed. AG Walton, Amsterdam: Elsevier pp. 273-289; Itakura et al., (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al., (1984) *Science* 198:1056; Ike et al., (1983) *Nucleic Acid Res.* 11:477). Such techniques have been employed in the directed evolution of other proteins (see, for example, Scott et al., (1990) *Science* 249:386-390; Roberts et al., (1992) *PNAS USA* 89:2429-2433; Devlin et al., (1990) *Science* 249: 404-406; Cwirla et al., (1990) *PNAS USA* 87: 6378-6382; as well as U.S. Patent Nos: 5,223,409, 5,198,346, and 5,096,815).

Alternatively, other forms of mutagenesis may be utilized to generate a combinatorial library. For example, protein homologs (both agonist and antagonist forms) may be generated and isolated from a library by screening using, for example, alanine scanning mutagenesis and the like (Ruf et al., (1994) *Biochemistry* 33:1565-1572; Wang et al., (1994) *J. Biol. Chem.* 269:3095-3099; Balint et al., (1993) *Gene* 137:109-118; Grodberg et al., (1993) *Eur. J. Biochem.* 218:597-601; Nagashima et al., (1993) *J. Biol. Chem.* 268:2888-2892; Lowman et al., (1991) *Biochemistry* 30:10832-10838; and Cunningham et al., (1989) *Science* 244:1081-1085), by linker scanning mutagenesis (Gustin et al., (1993) *Virology* 193:653-660; Brown et al., (1992) *Mol. Cell Biol.* 12:2644-2652; McKnight et al.,

(1982) *Science* 232:316); by saturation mutagenesis (Meyers et al., (1986) *Science* 232:613); by PCR mutagenesis (Leung et al., (1989) *Method Cell Mol Biol* 1:11-19); or by random mutagenesis (Miller et al., (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, NY; and Greener et al., (1994) *Strategies in Mol Biol* 7:32-34).

5 Linker scanning mutagenesis, particularly in a combinatorial setting, is an attractive method for identifying truncated forms of proteins that are bioactive.

A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations and truncations, and for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of protein homologs. The most widely used techniques for screening large gene libraries typically comprises cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Each of the illustrative assays described below are amenable to high throughput analysis as necessary to screen large numbers of degenerate sequences created by combinatorial mutagenesis techniques.

In an illustrative embodiment of a screening assay, candidate combinatorial gene products are displayed on the surface of a cell and the ability of particular cells or viral particles to bind to the combinatorial gene product is detected in a "panning assay". For instance, the gene library may be cloned into the gene for a surface membrane protein of a bacterial cell (Ladner et al., WO 88/06630; Fuchs et al., (1991) *Bio/Technology* 9:1370-1371; and Goward et al., (1992) *TIBS* 18:136-140), and the resulting fusion protein detected by panning, e.g. using a fluorescently labeled molecule which binds the cell surface protein, e.g. FITC-substrate, to score for potentially functional homologs. Cells may be visually inspected and separated under a fluorescence microscope, or, when the morphology of the cell permits, separated by a fluorescence-activated cell sorter. This method may be used to identify substrates or other polypeptides that can interact with a polypeptide of the invention.

In similar fashion, the gene library may be expressed as a fusion protein on the surface of a viral particle. For instance, in the filamentous phage system, foreign peptide sequences may be expressed on the surface of infectious phage, thereby conferring two

benefits. First, because these phage may be applied to affinity matrices at very high concentrations, a large number of phage may be screened at one time. Second, because each infectious phage displays the combinatorial gene product on its surface, if a particular phage is recovered from an affinity matrix in low yield, the phage may be amplified by another round of infection. The group of almost identical *E. coli* filamentous phages M13, fd, and f1 are most often used in phage display libraries, as either of the phage gIII or gVIII coat proteins may be used to generate fusion proteins without disrupting the ultimate packaging of the viral particle (Ladner et al., PCT publication WO 90/02909; Garrard et al., PCT publication WO 92/09690; Marks et al., (1992) *J. Biol. Chem.* 267:16007-16010; Griffiths et al., (1993) *EMBO J.* 12:725-734; Clackson et al., (1991) *Nature* 352:624-628; and Barbas et al., (1992) *PNAS USA* 89:4457-4461). Other phage coat proteins may be used as appropriate.

The invention also provides for reduction of the polypeptides of the invention to generate mimetics, e.g. peptide or non-peptide agents, which are able to mimic binding of the authentic protein to another cellular partner. Such mutagenic techniques as described above, as well as the thioredoxin system, are also particularly useful for mapping the determinants of a protein which participates in a protein-protein interaction with another protein. To illustrate, the critical residues of a protein which are involved in molecular recognition of a substrate protein may be determined and used to generate peptidomimetics that may bind to the substrate protein. The peptidomimetic may then be used as an inhibitor of the wild-type protein by binding to the substrate and covering up the critical residues needed for interaction with the wild-type protein, thereby preventing interaction of the protein and the substrate. By employing, for example, scanning mutagenesis to map the amino acid residues of a protein which are involved in binding a substrate polypeptide, peptidomimetic compounds may be generated which mimic those residues in binding to the substrate. For instance, non-hydrolyzable peptide analogs of such residues may be generated using benzodiazepine (e.g., see Freidinger et al., in *Peptides: Chemistry and Biology*, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), azepine (e.g., see Huffman et al., in *Peptides: Chemistry and Biology*, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), substituted gamma lactam rings (Garvey et al., in *Peptides: Chemistry and Biology*, G.R. Marshall ed., ESCOM Publisher: Leiden, Netherlands, 1988), keto-methylene pseudopeptides (Ewenson et al., (1986) *J. Med. Chem.* 29:295; and Ewenson et al., in *Peptides: Structure and Function* (Proceedings of the 9th

American Peptide Symposium) Pierce Chemical Co. Rockland, IL, 1985),  $\beta$ -turn dipeptide cores (Nagai et al., (1985) *Tetrahedron Lett* 26:647; and Sato et al., (1986) *J Chem Soc Perkin Trans* 1:1231), and  $\beta$ -aminoalcohols (Gordon et al., (1985) *Biochem Biophys Res Commun* 126:419; and Dann et al., (1986) *Biochem Biophys Res Commun* 134:71).

5       The activity of a polypeptide of the invention may be identified and/or assayed using a variety of methods well known to the skilled artisan. For example, information about the activity of non-essential genes may be assayed by creating a null mutant strain of bacteria expressing a mutant form of, or lacking expression of, a protein of interest. The resulting phenotype of the null mutant strain may provide information about the activity of  
10      the mutated gene product. Essential genes may be studied by creating a bacterial strain with a conditional mutation in the gene of interest. The bacterial strain may be grown under permissive and non-permissive conditions and the change in phenotype under the non-permissive conditions may be used to identify and/or assay the activity of the gene product.

15      In an alternative embodiment, the activity of a protein may be assayed using an appropriate substrate or binding partner or other reagent suitable to test for the suspected activity. For catalytic activity, the assay is typically designed so that the enzymatic reaction produces a detectable signal. For example, mixture of a kinase with a substrate in the presence of  $^{32}$ P will result in incorporation of the  $^{32}$ P into the substrate. The labeled  
20      substrate may then be separated from the free  $^{32}$ P and the presence and/or amount of radiolabeled substrate may be detected using a scintillation counter or a phosphorimager. Similar assays may be designed to identify and/or assay the activity of a wide variety of enzymatic activities. Based on the teachings herein, the skilled artisan would readily be able to develop an appropriate assay for a polypeptide of the invention.

25      In another embodiment, the activity of a polypeptide of the invention may be determined by assaying for the level of expression of RNA and/or protein molecules. Transcription levels may be determined, for example, using Northern blots, hybridization to an oligonucleotide array or by assaying for the level of a resulting protein product. Translation levels may be determined, for example, using Western blotting or by  
30      identifying a detectable signal produced by a protein product (e.g., fluorescence, luminescence, enzymatic activity, etc.). Depending on the particular situation, it may be desirable to detect the level of transcription and/or translation of a single gene or of multiple genes.

Alternatively, it may be desirable to measure the overall rate of DNA replication, transcription and/or translation in a cell. In general this may be accomplished by growing the cell in the presence of a detectable metabolite which is incorporated into the resultant DNA, RNA, or protein product. For example, the rate of DNA synthesis may be

5 determined by growing cells in the presence of BrdU which is incorporated into the newly synthesized DNA. The amount of BrdU may then be determined histochemically using an anti-BrdU antibody.

In general, the biological activity of a polypeptide encoded by SEQ ID NO. 2, and possibly other polypeptides of the invention, is peptidyl-tRNA hydrolase, having the gene

10 designation of *pth*. The polypeptide encoded by SEQ ID NO. 2, and possibly other polypeptides of the invention, may be further characterized as being part of the COG category "translation, ribosomal structure and biogenesis", with COG ID No. COG0193. The foregoing annotations were determined in accordance with the procedure described in EXAMPLE 17. This functionality assignment has been confirmed by completion of the X-ray structure of a polypeptide of the invention, as described in more detail below. In one aspect, the present invention contemplates a polypeptide having biological activity, or is a component of a protein complex having biological activity, substantially similar to or identical to peptidyl-tRNA hydrolase. Alternatively, the polypeptide catalyzes, or is a component of a protein complex that catalyzes, a reaction that is substantially the same type

15 of, or is the same as, the reaction catalyzed by peptidyl-tRNA hydrolase. Other biological activities of polypeptides of the invention are described herein, or will be reasonably apparent to those skilled in the art in light of the present disclosure.

During the elongation of a protein, peptidyl-tRNAs can dissociate prematurely from the ribosome, resulting in abortive polypeptide chain termination whereby peptidyl-tRNA

20 dissociates from the ribosome before the hydrolysis of the ester bond ("drop-off"). By cleaving the ester bond linking the tRNA and nascent peptide, peptidyl-tRNA hydrolase (PTH) works to ensure the recycling of all peptidyl-tRNAs produced through the abortion of the translation allowing the tRNA to be charged by the cognate aminoacyl-tRNA synthetase and then reutilized in protein synthesis. Initiator formyl-methionyl-tRNA<sub>f</sub><sup>MET</sup> is

25 the only aminoacyl-tRNA that is known to be safe from attack by PTH, thus enabling the cell to keep this tRNA intact for translation initiation.

PTH appears to be ubiquitous and has been shown to be essential for growth in *E. coli*, suggesting that its homolog in *P. aeruginosa* is also a potential molecular

therapeutic target for the development of antibacterial agents and other therapeutics. This enzymatic activity is essential for cell viability. In the absence of PTH activity, accumulation of peptidyl-tRNA species in the cell is rapid and protein synthesis becomes inhibited due to the lack of essential tRNA isoacceptors, leading to an inhibition of protein synthesis and eventually to cell death. Expression of very short open reading frames called mini-genes also lead to the inhibition of protein synthesis and an arrest in cell growth because peptidyl-tRNA hydrolase fails to recycle sufficiently rapidly peptidyl-tRNA released from ribosomes at the stop signal in competition with normal termination, causing starvation for essential species of tRNA. PTHs have also been recently suggested as facilitator of the splicing in vivo of the largest of the known catalytic RNAs (group II intron) in chloroplasts. Also, erythromycin, carbomycin, and lincosamide antibiotics are believed to function in part by stimulating the dissociation of peptidyl-tRNA from ribosomes, resulting in cell death.

The location of the PTH active site has been proposed to be similar to the one of the zinc-dependant aminopeptidase. The structural superimposition between *E. coli* PTH and the aminopeptidase together with *E. coli* PTH active site mapping has been determinant in a more precise identification of residues important for PTH activity. The three residues N10, H20 and D93 appear to be involved in the enzyme action. The catalytic efficiency of the H20A, N10A and D93A mutants were decreased by a factor of >100 while the K<sub>m</sub> value of the substrate (di-acetyl-Lys-tRNA<sup>Lys</sup>) remained unchanged in the case of the N10A mutation, and was increased by a factor of 1.6 in the case of D93A. Those residues make part of a channel at the surface of the enzyme where most of the strictly conserved amino-acids are located.

Site-directed mutagenesis has also been used to identify residues involved in binding of the tRNA moiety of the substrate. The position at the surface of PTH of the 3'-end of peptidyl-tRNAs corresponds to a four asparagines cluster (N10, N21, N68 and N114) located in the catalytic crevice. The receptor site of the 5'-phosphate of peptidyl-tRNAs possibly involves two cationic residues (R133 and K105) bordering each side of the channel. In the *E. coli* PTH, this channel is partially occupied by the C-end of a neighboring molecule. The binding of the three last residues of one PTH molecule to another PTH molecule is proposed to correspond to a product complex of the hydrolase that resembles to a tri-peptide esterified to its tRNA.

An alignment of amino acid sequences of *pth* from 6 pathogens is shown in FIGURE 10, and discussed in greater detail below.

For all of the foregoing reasons, the polypeptides of the present invention are potentially valuable targets for therapeutics and diagnostics.

5

### 3. Nucleic Acids of the Invention

One aspect of the invention pertains to isolated nucleic acids of the invention. For example, the present invention contemplates an isolated nucleic acid comprising (a) the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 3, (b) a nucleotide sequence at least

10 80% identical to SEQ ID NO: 1 or SEQ ID NO: 3, (c) a nucleotide sequence that hybridizes under stringent conditions to SEQ ID NO: 1 or SEQ ID NO: 3, or (d) the complement of the nucleotide sequence of (a), (b) or (c). In certain embodiments, nucleic acids of the invention may be labeled, with for example, a radioactive, chemiluminescent or fluorescent label.

15

It may be that case that the nucleic acid sequence of SEQ ID NO: 3 differs from that of SEQ ID NO: 1 by one or more nucleic acid residues. SEQ ID NO: 3 is determined experimentally, and SEQ ID NO: 1 obtained as described in EXAMPLE 1. In such a case, the present invention contemplates the specific nucleic acid sequences of SEQ ID NO: 1 and SEQ ID NO: 3, and variants thereof, as well as any differences in the applicable amino acid sequences encoded thereby.

20

In another aspect, the present invention contemplates an isolated nucleic acid that specifically hybridizes under stringent conditions to at least ten nucleotides of SEQ ID NO: 1 or SEQ ID NO: 3, or the complement thereof, which nucleic acid can specifically detect or amplify SEQ ID NO: 1 or SEQ ID NO: 3, or the complement thereof. In yet another

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aspect, the present invention contemplates such an isolated nucleic acid comprising a nucleotide sequence encoding a fragment of SEQ ID NO: 2 or SEQ ID NO: 4 at least 8 residues in length. The present invention further contemplates a method of hybridizing an oligonucleotide with a nucleic acid of the invention comprising: (a) providing a single-stranded oligonucleotide at least eight nucleotides in length, the oligonucleotide being

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complementary to a portion of a nucleic acid of the invention; and (b) contacting the oligonucleotide with a sample comprising a nucleic acid of the acid under conditions that permit hybridization of the oligonucleotide with the nucleic acid of the invention.

Isolated nucleic acids which differ from the nucleic acids of the invention due to degeneracy in the genetic code are also within the scope of the invention. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) 5 may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the polypeptides of the invention will exist. One skilled in the art will appreciate that these variations in one or more nucleotides (from less than 1% up to about 3 or 5% or possibly more of the nucleotides) of the nucleic acids encoding a 10 particular protein of the invention may exist among a given species due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of this invention.

Bias in codon choice within genes in a single species appears related to the level of expression of the protein encoded by that gene. Accordingly, the invention encompasses 15 nucleic acid sequences which have been optimized for improved expression in a host cell by altering the frequency of codon usage in the nucleic acid sequence to approach the frequency of preferred codon usage of the host cell. Due to codon degeneracy, it is possible to optimize the nucleotide sequence without affecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleotide sequence 20 that encodes all or a substantial portion of the amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 4 or other polypeptides of the invention.

The present invention pertains to nucleic acids encoding proteins derived from *P. aeruginosa* and which have amino acid sequences evolutionarily related to a polypeptide of the invention, wherein "evolutionarily related to", refers to proteins having different amino 25 acid sequences which have arisen naturally (e.g. by allelic variance or by differential splicing), as well as mutational variants of the proteins of the invention which are derived, for example, by combinatorial mutagenesis.

Fragments of the polynucleotides of the invention encoding a biologically active portion of the subject polypeptides are also within the scope of the invention. As used 30 herein, a fragment of a nucleic acid of the invention encoding an active portion of a polypeptide of the invention refers to a nucleotide sequence having fewer nucleotides than the nucleotide sequence encoding the full length amino acid sequence of a polypeptide of the invention, for example, SEQ ID NO: 2 or SEQ ID NO: 4, and which encodes a

polypeptide which retains at least a portion of a biological activity of the full-length protein as defined herein, or alternatively, which is functional as a modulator of the biological activity of the full-length protein. For example, such fragments include a polypeptide containing a domain of the full-length protein from which the polypeptide is derived that

5 mediates the interaction of the protein with another molecule (e.g., polypeptide, DNA, RNA, etc.). In another embodiment, the present invention contemplates an isolated nucleic acid that encodes a polypeptide having a biological activity of a protein having the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, or alternatively biological activity of peptidyl-tRNA hydrolase.

10 Nucleic acids within the scope of the invention may also contain linker sequences, modified restriction endonuclease sites and other sequences useful for molecular cloning, expression or purification of such recombinant polypeptides.

A nucleic acid encoding a polypeptide of the invention may be obtained from mRNA or genomic DNA from any organism in accordance with protocols described herein, 15 as well as those generally known to those skilled in the art. A cDNA encoding a polypeptide of the invention, for example, may be obtained by isolating total mRNA from an organism, e.g. a bacteria, virus, mammal, etc. Double stranded cDNAs may then be prepared from the total mRNA, and subsequently inserted into a suitable plasmid or bacteriophage vector using any one of a number of known techniques. A gene encoding a 20 polypeptide of the invention may also be cloned using established polymerase chain reaction techniques in accordance with the nucleotide sequence information provided by the invention. In one aspect, the present invention contemplates a method for amplification of a nucleic acid of the invention, or a fragment thereof, comprising: (a) providing a pair of single stranded oligonucleotides, each of which is at least eight nucleotides in length, 25 complementary to sequences of a nucleic acid of the invention, and wherein the sequences to which the oligonucleotides are complementary are at least ten nucleotides apart; and (b) contacting the oligonucleotides with a sample comprising a nucleic acid comprising the nucleic acid of the invention under conditions which permit amplification of the region located between the pair of oligonucleotides, thereby amplifying the nucleic acid.

30 Another aspect of the invention relates to the use of nucleic acids of the invention in "antisense therapy". As used herein, antisense therapy refers to administration or *in situ* generation of oligonucleotide probes or their derivatives which specifically hybridize or otherwise bind under cellular conditions with the cellular mRNA and/or genomic DNA

encoding one of the polypeptides of the invention so as to inhibit expression of that polypeptide, e.g. by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general,

5 antisense therapy refers to the range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.

An antisense construct of the present invention may be delivered, for example, as an expression plasmid which, when transcribed in the cell, produces RNA which is complementary to at least a unique portion of the mRNA which encodes a polypeptide of

10 the invention. Alternatively, the antisense construct may be an oligonucleotide probe which is generated *ex vivo* and which, when introduced into the cell causes inhibition of expression by hybridizing with the mRNA and/or genomic sequences encoding a polypeptide of the invention. Such oligonucleotide probes may be modified oligonucleotides which are resistant to endogenous nucleases, e.g. exonucleases and/or  
15 endonucleases, and are therefore stable *in vivo*. Exemplary nucleic acid molecules for use as antisense oligonucleotides are phosphoramidate, phosphothioate and methylphosphonate analogs of DNA (see also U.S. Patents 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy have been reviewed, for example, by van der Krol et al., (1988) *Biotechniques* 6:958-976; and Stein et  
20 al., (1988) *Cancer Res* 48:2659-2668.

In a further aspect, the invention provides double stranded small interfering RNAs (siRNAs), and methods for administering the same. siRNAs decrease or block gene expression. While not wishing to be bound by theory, it is generally thought that siRNAs inhibit gene expression by mediating sequence specific mRNA degradation. RNA

25 interference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing, particularly in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene (Elbashir et al. *Nature* 2001; 411(6836): 494-8). Accordingly, it is understood that siRNAs and long dsRNAs having substantial sequence identity to all or a portion of SEQ ID NO: 1 or SEQ ID NO: 3 may be used to  
30 inhibit the expression of a nucleic acid of the invention, and particularly when the polynucleotide is expressed in a mammalian or plant cell.

The nucleic acids of the invention may be used as diagnostic reagents to detect the presence or absence of the target DNA or RNA sequences to which they specifically bind,

such as for determining the level of expression of a nucleic acid of the invention. In one aspect, the present invention contemplates a method for detecting the presence of a nucleic acid of the invention or a portion thereof in a sample, the method comprising: (a) providing an oligonucleotide at least eight nucleotides in length, the oligonucleotide being complementary to a portion of a nucleic acid of the invention; (b) contacting the oligonucleotide with a sample comprising at least one nucleic acid under conditions that permit hybridization of the oligonucleotide with a nucleic acid comprising a nucleotide sequence complementary thereto; and (c) detecting hybridization of the oligonucleotide to a nucleic acid in the sample, thereby detecting the presence of a nucleic acid of the invention or a portion thereof in the sample. In another aspect, the present invention contemplates a method for detecting the presence of a nucleic acid of the invention or a portion thereof in a sample, the method comprising: (a) providing a pair of single stranded oligonucleotides, each of which is at least eight nucleotides in length, complementary to sequences of a nucleic acid of the invention, and wherein the sequences to which the oligonucleotides are complementary are at least ten nucleotides apart; and (b) contacting the oligonucleotides with a sample comprising at least one nucleic acid under hybridization conditions; (c) amplifying the nucleotide sequence between the two oligonucleotide primers; and (d) detecting the presence of the amplified sequence, thereby detecting the presence of a nucleic acid comprising the nucleic acid of the invention or a portion thereof in the sample.

In another aspect of the invention, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding a polypeptide of the invention and operably linked to at least one regulatory sequence. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. The vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should be considered.

The subject nucleic acids may be used to cause expression and over-expression of a polypeptide of the invention in cells propagated in culture, e.g. to produce proteins or polypeptides, including fusion proteins or polypeptides.

This invention pertains to a host cell transfected with a recombinant gene in order to express a polypeptide of the invention. The host cell may be any prokaryotic or eukaryotic cell. For example, a polypeptide of the invention may be expressed in bacterial cells, such as *E. coli*, insect cells (baculovirus), yeast, or mammalian cells. In those instances when the

host cell is human, it may or may not be in a live subject. Other suitable host cells are known to those skilled in the art. Additionally, the host cell may be supplemented with tRNA molecules not typically found in the host so as to optimize expression of the polypeptide. Other methods suitable for maximizing expression of the polypeptide will be 5 known to those in the art.

The present invention further pertains to methods of producing the polypeptides of the invention. For example, a host cell transfected with an expression vector encoding a polypeptide of the invention may be cultured under appropriate conditions to allow expression of the polypeptide to occur. The polypeptide may be secreted and isolated from 10 a mixture of cells and medium containing the polypeptide. Alternatively, the polypeptide may be retained cytoplasmically and the cells harvested, lysed and the protein isolated.

A cell culture includes host cells, media and other byproducts. Suitable media for cell culture are well known in the art. The polypeptide may be isolated from cell culture medium, host cells, or both using techniques known in the art for purifying proteins, 15 including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, and immunoaffinity purification with antibodies specific for particular epitopes of a polypeptide of the invention.

Thus, a nucleotide sequence encoding all or a selected portion of polypeptide of the invention, may be used to produce a recombinant form of the protein via microbial or 20 eukaryotic cellular processes. Ligating the sequence into a polynucleotide construct, such as an expression vector, and transforming or transfecting into hosts, either eukaryotic (yeast, avian, insect or mammalian) or prokaryotic (bacterial cells), are standard procedures. Similar procedures, or modifications thereof, may be employed to prepare recombinant polypeptides of the invention by microbial means or tissue-culture technology.

25 Expression vehicles for production of a recombinant protein include plasmids and other vectors. For instance, suitable vectors for the expression of a polypeptide of the invention include plasmids of the types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

30 A number of vectors exist for the expression of recombinant proteins in yeast. For instance, YEP24, YIP5, YEP51, YEP52, pYES2, and YRP17 are cloning and expression vehicles useful in the introduction of genetic constructs into *S. cerevisiae* (see, for example, Broach et al., (1983) in *Experimental Manipulation of Gene Expression*, ed. M. Inouye

Academic Press, p. 83). These vectors may replicate in *E. coli* due the presence of the pBR322 ori, and in *S. cerevisiae* due to the replication determinant of the yeast 2 micron plasmid. In addition, drug resistance markers such as ampicillin may be used.

In certain embodiments, mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see *Molecular Cloning A Laboratory Manual*, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 1989) Chapters 16 and 17. In some instances, it may be desirable to express the recombinant protein by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the β-gal containing pBlueBac III).

In another variation, protein production may be achieved using *in vitro* translation systems. *In vitro* translation systems are, generally, a translation system which is a cell-free extract containing at least the minimum elements necessary for translation of an RNA molecule into a protein. An *in vitro* translation system typically comprises at least ribosomes, tRNAs, initiator methionyl-tRNAMet, proteins or complexes involved in translation, e.g., eIF2, eIF3, the cap-binding (CB) complex, comprising the cap-binding protein (CBP) and eukaryotic initiation factor 4F (eIF4F). A variety of *in vitro* translation systems are well known in the art and include commercially available kits. Examples of *in vitro* translation systems include eukaryotic lysates, such as rabbit reticulocyte lysates, rabbit oocyte lysates, human cell lysates, insect cell lysates and wheat germ extracts. Lysates are commercially available from manufacturers such as Promega Corp., Madison,

Wis.; Stratagene, La Jolla, Calif.; Amersham, Arlington Heights, Ill.; and GIBCO/BRL, Grand Island, N.Y. *In vitro* translation systems typically comprise macromolecules, such as enzymes, translation, initiation and elongation factors, chemical reagents, and ribosomes. In addition, an *in vitro* transcription system may be used. Such systems typically comprise 5 at least an RNA polymerase holoenzyme, ribonucleotides and any necessary transcription initiation, elongation and termination factors. *In vitro* transcription and translation may be coupled in a one-pot reaction to produce proteins from one or more isolated DNAs.

When expression of a carboxy terminal fragment of a polypeptide is desired, i.e. a truncation mutant, it may be necessary to add a start codon (ATG) to the oligonucleotide 10 fragment containing the desired sequence to be expressed. It is well known in the art that a methionine at the N-terminal position may be enzymatically cleaved by the use of the enzyme methionine aminopeptidase (MAP). MAP has been cloned from *E. coli* (Ben-Bassat et al., (1987) *J. Bacteriol.* 169:751-757) and *Salmonella typhimurium* and its *in vitro* activity has been demonstrated on recombinant proteins (Miller et al., (1987) *PNAS USA* 15 84:2718-1722). Therefore, removal of an N-terminal methionine, if desired, may be achieved either *in vivo* by expressing such recombinant polypeptides in a host which produces MAP (e.g., *E. coli* or CM89 or *S. cerevisiae*), or *in vitro* by use of purified MAP (e.g., procedure of Miller et al.).

Coding sequences for a polypeptide of interest may be incorporated as a part of a 20 fusion gene including a nucleotide sequence encoding a different polypeptide. The present invention contemplates an isolated nucleic acid comprising a nucleic acid of the invention and at least one heterologous sequence encoding a heterologous peptide linked in frame to the nucleotide sequence of the nucleic acid of the invention so as to encode a fusion protein comprising the heterologous polypeptide. The heterologous polypeptide may be fused to 25 (a) the C-terminus of the polypeptide encoded by the nucleic acid of the invention, (b) the N-terminus of the polypeptide, or (c) the C-terminus and the N-terminus of the polypeptide. In certain instances, the heterologous sequence encodes a polypeptide permitting the detection, isolation, solubilization and/or stabilization of the polypeptide to which it is fused. In still other embodiments, the heterologous sequence encodes a polypeptide 30 selected from the group consisting of a polyHis tag, myc, HA, GST, protein A, protein G, calmodulin-binding peptide, thioredoxin, maltose-binding protein, poly arginine, poly His-Asp, FLAG, a portion of an immunoglobulin protein, and a transcytosis peptide.

Fusion expression systems can be useful when it is desirable to produce an immunogenic fragment of a polypeptide of the invention. For example, the VP6 capsid protein of rotavirus may be used as an immunologic carrier protein for portions of polypeptide, either in the monomeric form or in the form of a viral particle. The nucleic acid sequences corresponding to the portion of a polypeptide of the invention to which antibodies are to be raised may be incorporated into a fusion gene construct which includes coding sequences for a late vaccinia virus structural protein to produce a set of recombinant viruses expressing fusion proteins comprising a portion of the protein as part of the virion. The Hepatitis B surface antigen may also be utilized in this role as well. Similarly, chimeric constructs coding for fusion proteins containing a portion of a polypeptide of the invention and the poliovirus capsid protein may be created to enhance immunogenicity (see, for example, EP Publication NO: 0259149; and Evans et al., (1989) *Nature* 339:385; Huang et al., (1988) *J. Virol.* 62:3855; and Schlienger et al., (1992) *J. Virol.* 66:2).

Fusion proteins may facilitate the expression and/or purification of proteins. For example, a polypeptide of the invention may be generated as a glutathione-S-transferase (GST) fusion protein. Such GST fusion proteins may be used to simplify purification of a polypeptide of the invention, such as through the use of glutathione-derivatized matrices (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al., (N.Y.: John Wiley & Sons, 1991)). In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant protein, may allow purification of the expressed fusion protein by affinity chromatography using a Ni<sup>2+</sup> metal resin. The purification leader sequence may then be subsequently removed by treatment with enterokinase to provide the purified protein (e.g., see Hochuli et al., (1987) *J. Chromatography* 411: 177; and Janknecht et al., *PNAS USA* 88:8972).

Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene may be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments may be carried out using anchor primers which give rise to

complementary overhangs between two consecutive gene fragments which may subsequently be annealed to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel et al., John Wiley & Sons: 1992).

5 The present invention further contemplates a transgenic non-human animal having cells which harbor a transgene comprising a nucleic acid of the invention.

In other embodiments, the invention provides for nucleic acids of the invention immobilized onto a solid surface, including, plates, microtiter plates, slides, beads, particles, spheres, films, strands, precipitates, gels, sheets, tubing, containers, capillaries, pads, slices, etc. The nucleic acids of the invention may be immobilized onto a chip as part 10 of an array. The array may comprise one or more polynucleotides of the invention as described herein. In one embodiment, the chip comprises one or more polynucleotides of the invention as part of an array of *P. aeruginosa* polynucleotide sequences.

In still other embodiments, the invention comprises the sequence of a nucleic acid of the invention in computer readable format. The invention also encompasses a database 15 comprising the sequence of a nucleic acid of the invention.

#### *4. Homology Searching of Nucleotide and Polypeptide Sequences*

The nucleotide or amino acid sequences of the invention, including those set forth in the appended Figures, may be used as query sequences against databases such as GenBank, 20 SwissProt, PDB, BLOCKS, and Pima II. These databases contain previously identified and annotated sequences that may be searched for regions of homology (similarity) using BLAST, which stands for Basic Local Alignment Search Tool (Altschul S F (1993) J Mol Evol 36:290-300; Altschul, S F et al (1990) J Mol Biol 215:403-10).

BLAST produces alignments of both nucleotide and amino acid sequences to 25 determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal or plant) origin. Other algorithms such as the one described in Smith, R. F. and T. F. Smith (1992; Protein Engineering 5:35-51) may be used when dealing with primary sequence patterns and secondary structure gap 30 penalties. In the usual course using BLAST, sequences have lengths of at least 49 nucleotides and no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach, as detailed in Karlin and Altschul (1993; Proc Nat Acad Sci 90:5873-7) searches matches between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The threshold is typically set at 5 about 10-25 for nucleotides and about 3-15 for peptides.

### 5. Analysis of Protein Properties

#### (a) Analysis of Proteins by Mass Spectrometry

Typically, protein characterization by mass spectroscopy first requires protein isolation followed by either chemical or enzymatic digestion of the protein into smaller peptide fragments, whereupon the peptide fragments may be analyzed by mass spectrometry to obtain a peptide map. Mass spectrometry may also be used to identify post-translational modifications (e.g., phosphorylation, etc.) of a polypeptide.

Various mass spectrometers may be used within the present invention. 15 Representative examples include: triple quadrupole mass spectrometers, magnetic sector instruments (magnetic tandem mass spectrometer, JEOL, Peabody, Mass), ionspray mass spectrometers (Bruins et al., Anal Chem. 59:2642-2647, 1987), electrospray mass spectrometers (including tandem, nano- and nano-electrospray tandem) (Fenn et al., Science 246:64-71, 1989), laser desorption time-of-flight mass spectrometers (Karas and Hillenkamp, Anal. Chem. 60:2299-2301, 1988), and a Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (Extrel Corp., Pittsburgh, Mass.).

MALDI ionization is a technique in which samples of interest, in this case peptides and proteins, are co-crystallized with an acidified matrix. The matrix is typically a small molecule that absorbs at a specific wavelength, generally in the ultraviolet (UV) range, and dissipates the absorbed energy thermally. Typically a pulsed laser beam is used to transfer energy rapidly (i.e., a few ns) to the matrix. This transfer of energy causes the matrix to rapidly dissociate from the MALDI plate surface and results in a plume of matrix and the co-crystallized analytes being transferred into the gas phase. MALDI is considered a "soft-ionization" method that typically results in singly-charged species in the gas phase, most often resulting from a protonation reaction with the matrix. MALDI may be coupled in-line with time of flight (TOF) mass spectrometers. TOF detectors are based on the principle that an analyte moves with a velocity proportional to its mass. Analytes of higher mass move slower than analytes of lower mass and thus reach the detector later than lighter

analytes. The present invention contemplates a composition comprising a polypeptide of the invention and a matrix suitable for mass spectrometry. In certain instances, the matrix is a nicotinic acid derivative or a cinnamic acid derivative.

MALDI-TOF MS is easily performed with modern mass spectrometers. Typically 5 the samples of interest, in this case peptides or proteins, are mixed with a matrix and spotted onto a polished stainless steel plate (MALDI plate). Commercially available MALDI plates can presently hold up to 1536 samples per plate. Once spotted with sample, the MALDI sample plate is then introduced into the vacuum chamber of a MALDI mass spectrometer. The pulsed laser is then activated and the mass to charge ratios of the 10 analytes are measured utilizing a time of flight detector. A mass spectrum representing the mass to charge ratios of the peptides/proteins is generated.

As mentioned above, MALDI can be utilized to measure the mass to charge ratios 15 of both proteins and peptides. In the case of proteins, a mixture of intact protein and matrix are co-crystallized on a MALDI target (Karas, M. and Hillenkamp, F. Anal. Chem. 1988, 60 (20) 2299-2301). The spectrum resulting from this analysis is employed to determine the molecular weight of a whole protein. This molecular weight can then be compared to the theoretical weight of the protein and utilized in characterizing the analyte of interest, such as whether or not the protein has undergone post-translational modifications (e.g., example phosphorylation).

In certain embodiments, MALDI mass spectrometry is used for determination of 20 peptide maps of digested proteins. The peptide masses are measured accurately using a MALDI-TOF or a MALDI-Q-Star mass spectrometer, with detection precision down to the low ppm (parts per million) level. The ensemble of the peptide masses observed in a protein digest, such as a tryptic digest, may be used to search protein/DNA databases in a 25 method called peptide mass fingerprinting. In this approach, protein entries in a database are ranked according to the number of experimental peptide masses that match the predicted trypsin digestion pattern. Commercially available software utilizes a search algorithm that provides a scoring scheme based on the size of the databases, the number of matching peptides, and the different peptides. Depending on the number of peptides 30 observed, the accuracy of the measurement, and the size of the genome of the particular species, unambiguous protein identification may be obtained.

Statistical analysis may be performed upon each protein match to determine the validity of the match. Typical constraints include error tolerances within 0.1 Da for

monoisotopic peptide masses, cysteines may be alkylated and searched as carboxyamidomethyl modifications, 0 or 1 missed enzyme cleavages, and no methionine oxidations allowed. Identified proteins may be stored automatically in a relational database with software links to SDS-PAGE images and ligand sequences. Often even a partial

5 peptide map is specific enough for identification of the protein. If no protein match is found, a more error-tolerant search can be used, for example using fewer peptides or allowing a larger margin error with respect to mass accuracy.

Other mass spectroscopy methods such as tandem mass spectrometry or post source decay may be used to obtain sequence information about proteins that cannot be identified  
10 by peptide mass mapping, or to confirm the identity of proteins that are tentatively identified by an error-tolerant peptide mass search described above. (Griffin et al, Rapid Commun. Mass. Spectrom. 1995, 9, 1546-51).

*(b) Analysis of Proteins by Nuclear Magnetic Resonance (NMR)*

NMR may be used to characterize the structure of a polypeptide in accordance with  
15 the methods of the invention. In particular, NMR can be used, for example, to determine the three dimensional structure, the conformational state, the aggregation level, the state of protein folding/unfolding or the dynamic properties of a polypeptide. For example, the present invention contemplates a method for determining three dimensional structure information of a polypeptide of the invention, the method comprising: (a) generating a  
20 purified isotopically labeled polypeptide of the invention; and (b) subjecting the polypeptide to NMR spectroscopic analysis, thereby determining information about its three dimensional structure.

Interaction between a polypeptide and another molecule can also be monitored  
25 using NMR. Thus, the invention encompasses methods for detecting, designing and characterizing interactions between a polypeptide and another molecule, including polypeptides, nucleic acids and small molecules, utilizing NMR techniques. For example, the present invention contemplates a method for determining three dimensional structure information of a polypeptide of the invention, or a fragment thereof, while the polypeptide is complexed with another molecule, the method comprising: (a) generating a purified  
30 isotopically labeled polypeptide of the invention, or a fragment thereof; (b) forming a complex between the polypeptide and the other molecule; and (c) subjecting the complex to NMR spectroscopic analysis, thereby determining information about the three dimensional structure of the polypeptide. In another aspect, the present invention contemplates a

method for identifying compounds that bind to a polypeptide of the invention, or a fragment thereof, the method comprising: (a) generating a first NMR spectrum of an isotopically labeled polypeptide of the invention, or a fragment thereof; (b) exposing the polypeptide to one or more chemical compounds; (c) generating a second NMR spectrum of the 5 polypeptide which has been exposed to one or more chemical compounds; and (d) comparing the first and second spectra to determine differences between the first and the second spectra, wherein the differences are indicative of one or more compounds that have bound to the polypeptide.

Briefly, the NMR technique involves placing the material to be examined (usually 10 in a suitable solvent) in a powerful magnetic field and irradiating it with radio frequency (rf) electromagnetic radiation. The nuclei of the various atoms will align themselves with the magnetic field until energized by the rf radiation. They then absorb this resonant energy and re-radiate it at a frequency dependent on i) the type of nucleus and ii) its atomic environment. Moreover, resonant energy may be passed from one nucleus to another, 15 either through bonds or through three-dimensional space, thus giving information about the environment of a particular nucleus and nuclei in its vicinity.

However, it is important to recognize that not all nuclei are NMR active. Indeed, not all isotopes of the same element are active. For example, whereas "ordinary" hydrogen, <sup>1</sup>H, is NMR active, heavy hydrogen (deuterium), <sup>2</sup>H, is not active in the same way. Thus, 20 any material that normally contains <sup>1</sup>H hydrogen may be rendered "invisible" in the hydrogen NMR spectrum by replacing all or almost all the <sup>1</sup>H hydrogens with <sup>2</sup>H. It is for this reason that NMR spectroscopic analyses of water-soluble materials frequently are performed in <sup>2</sup>H<sub>2</sub>O (or deuterium) to eliminate the water signal.

Conversely, "ordinary" carbon, <sup>12</sup>C, is NMR inactive whereas the stable isotope, 25 <sup>13</sup>C, present to about 1% of total carbon in nature, is active. Similarly, while "ordinary" nitrogen, <sup>14</sup>N, is NMR active, it has undesirable properties for NMR and resonates at a different frequency from the stable isotope <sup>15</sup>N, present to about 0.4% of total nitrogen in nature.

By labeling proteins with <sup>15</sup>N and <sup>15</sup>N/<sup>13</sup>C, it is possible to conduct analytical NMR 30 of macromolecules with weights of 15 kD and 40 kD, respectively. More recently, partial deuteration of the protein in addition to <sup>13</sup>C- and <sup>15</sup>N-labeling has increased the possible weight of proteins and protein complexes for NMR analysis still further, to approximately 60-70 kD. See Shan et al., J. Am. Chem.Soc., 118:6570-6579 (1996); L.E. Kay, Methods

Enzymol., 339:174-203 (2001); and K.H. Gardner & L.E. Kay, Annu Rev Biophys Biomol Struct., 27:357-406 (1998); and references cited therein.

Isotopic substitution may be accomplished by growing a bacterium or yeast or other type of cultured cells, transformed by genetic engineering to produce the protein of choice,

5 in a growth medium containing  $^{13}\text{C}$ -,  $^{15}\text{N}$ - and/or  $^2\text{H}$ -labeled substrates. In certain instances, bacterial growth media consists of  $^{13}\text{C}$ -labeled glucose and/or  $^{15}\text{N}$ -labeled ammonium salts dissolved in  $\text{D}_2\text{O}$  where necessary. Kay, L. et al., Science, 249:411 (1990) and references therein and Bax, A., J. Am. Chem. Soc., 115, 4369 (1993). More recently, isotopically labeled media especially adapted for the labeling of bacterially produced

10 macromolecules have been described. See U.S. Pat. No. 5,324,658.

The goal of these methods has been to achieve universal and/or random isotopic enrichment of all of the amino acids of the protein. By contrast, other methods allow only certain residues to be relatively enriched in  $^1\text{H}$ ,  $^2\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$ . For example, Kay et al., J. Mol. Biol., 263, 627-636 (1996) and Kay et al., J. Am. Chem. Soc., 119, 7599-7600 (1997)

15 have described methods whereby isoleucine, alanine, valine and leucine residues in a protein may be labeled with  $^2\text{H}$ ,  $^{13}\text{C}$  and  $^{15}\text{N}$ , and may be specifically labeled with  $^1\text{H}$  at the terminal methyl position. In this way, study of the proton-proton interactions between some amino acids may be facilitated. Similarly, a cell-free system has been described by Yokoyama et al., J. Biomol. NMR, 6(2), 129-134 (1995), wherein a transcription-

20 translation system derived from *E. coli* was used to express human Ha-Ras protein incorporating  $^{15}\text{N}$  into serine and/or aspartic acid.

Techniques for producing isotopically labeled proteins and macromolecules, such as glycoproteins, in mammalian or insect cells have been described. See U.S. Pat. Nos. 5,393,669 and 5,627,044; Weller, C. T., Biochem., 35, 8815-23 (1996) and Lustbader, J. W., J. Biomol. NMR, 7, 295-304 (1996). Other methods for producing polypeptides and other molecules with labels appropriate for NMR are known in the art.

The present invention contemplates using a variety of solvents which are appropriate for NMR. For  $^1\text{H}$  NMR, a deuterium lock solvent may be used. Exemplary deuterium lock solvents include acetone ( $\text{CD}_3\text{COCD}_3$ ), chloroform ( $\text{CDCl}_3$ ), dichloro

30 methane ( $\text{CD}_2\text{Cl}_2$ ), methylnitrile ( $\text{CD}_3\text{CN}$ ), benzene ( $\text{C}_6\text{D}_6$ ), water ( $\text{D}_2\text{O}$ ), diethylether ( $(\text{CD}_3\text{CD}_2)_2\text{O}$ ), dimethylether ( $(\text{CD}_3)_2\text{O}$ ), N,N-dimethylformamide ( $(\text{CD}_3)_2\text{NCDO}$ ), dimethyl sulfoxide ( $\text{CD}_3\text{SOCD}_3$ ), ethanol ( $\text{CD}_3\text{CD}_2\text{OD}$ ), methanol ( $\text{CD}_3\text{OD}$ ), tetrahydrofuran ( $\text{C}_4\text{D}_8\text{O}$ ), toluene ( $\text{C}_6\text{D}_5\text{CD}_3$ ), pyridine ( $\text{C}_5\text{D}_5\text{N}$ ) and cyclohexane ( $\text{C}_6\text{H}_{12}$ ).

For example, the present invention contemplates a composition comprising a polypeptide of the invention and a deuterium lock solvent.

The 2-dimensional  $^1\text{H}$ - $^{15}\text{N}$  HSQC (Heteronuclear Single Quantum Correlation) spectrum provides a diagnostic fingerprint of conformational state, aggregation level, state 5 of protein folding, and dynamic properties of a polypeptide (Yee et al, PNAS 99, 1825-30 (2002)). Polypeptides in aqueous solution usually populate an ensemble of 3-dimensional structures which can be determined by NMR. When the polypeptide is a stable globular protein or domain of a protein, then the ensemble of solution structures is one of very closely related conformations. In this case, one peak is expected for each non-proline 10 residue with a dispersion of resonance frequencies with roughly equal intensity. Additional pairs of peaks from side-chain  $\text{NH}_2$  groups are also often observed, and correspond to the approximate number of Gln and Asn residues in the protein. This type of HSQC spectra usually indicates that the protein is amenable to structure determination by NMR methods.

If the HSQC spectrum shows well-dispersed peaks but there are either too few or 15 too many in number, and/or the peak intensities differ throughout the spectrum, then the protein likely does not exist in a single globular conformation. Such spectral features are indicative of conformational heterogeneity with slow or nonexistent inter-conversion between states (too many peaks) or the presence of dynamic processes on an intermediate timescale that can broaden and obscure the NMR signals. Proteins with this type of 20 spectrum can sometimes be stabilized into a single conformation by changing either the protein construct, the solution conditions, temperature or by binding of another molecule.

The  $^1\text{H}$ - $^{15}\text{N}$  HSQC can also indicate whether a protein has formed large nonspecific aggregates or has dynamic properties. Alternatively, proteins that are largely unfolded, e.g., having very little regular secondary structure, result in  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra in which the 25 peaks are all very narrow and intense, but have very little spectral dispersion in the  $^{15}\text{N}$ -dimension. This reflects the fact that many or most of the amide groups of amino acids in unfolded polypeptides are solvent exposed and experience similar chemical environments resulting in similar  $^1\text{H}$  chemical shifts.

The use of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC, can thus allow the rapid characterization of the 30 conformational state, aggregation level, state of protein folding, and dynamic properties of a polypeptide. Additionally, other 2D spectra such as  $^1\text{H}$ - $^{13}\text{C}$  HSQC, or HNCO spectra can also be used in a similar manner. Further use of the  $^1\text{H}$ - $^{15}\text{N}$  HSQC combined with relaxation measurements can reveal the molecular rotational correlation time and dynamic

properties of polypeptides. The rotational correlation time is proportional to size of the protein and therefore can reveal if it forms specific homo-oligomers such as homodimers, homotetramers, etc.

The structure of stable globular proteins can be determined through a series of well-described procedures. For a general review of structure determination of globular proteins in solution by NMR spectroscopy, see Wüthrich, *Science* 243: 45-50 (1989). See also, Billeter et al., *J. Mol. Biol.* 155: 321-346 (1982). Current methods for structure determination usually require the complete or nearly complete sequence-specific assignment of <sup>1</sup>H-resonance frequencies of the protein and subsequent identification of approximate inter-hydrogen distances (from nuclear Overhauser effect (NOE) spectra) for use in restrained molecular dynamics calculations of the protein conformation. One approach for the analysis of NMR resonance assignments was first outlined by Wüthrich, Wagner and co-workers (Wüthrich, "NMR of proteins and nucleic acids" Wiley, New York, New York (1986); Wüthrich, *Science* 243: 45-50 (1989); Billeter et al., *J. Mol. Biol.* 155: 321-346 (1982)). Newer methods for determining the structures of globular proteins include the use of residual dipolar coupling restraints (Tian et al., *J Am Chem Soc.* 2001 Nov 28;123(47):11791-6; Bax et al, *Methods Enzymol.* 2001;339:127-74) and empirically derived conformational restraints (Zweckstetter & Bax, *J Am Chem Soc.* 2001 Sep 26;123(38):9490-1). It has also been shown that it may be possible to determine structures of globular proteins using only un-assigned NOE measurements. NMR may also be used to determine ensembles of many inter-converting, unfolded conformations (Choy and Forman-Kay, *J Mol Biol.* 2001 May 18;308(5):1011-32).

NMR analysis of a polypeptide in the presence and absence of a test compound (e.g., a polypeptide, nucleic acid or small molecule) may be used to characterize interactions between a polypeptide and another molecule. Because the <sup>1</sup>H-<sup>15</sup>N HSQC spectrum and other simple 2D NMR experiments can be obtained very quickly (on the order of minutes depending on protein concentration and NMR instrumentation), they are very useful for rapidly testing whether a polypeptide is able to bind to another molecule. Changes in the resonance frequency (in one or both dimensions) of one or more peaks in the HSQC spectrum indicate an interaction with another molecule. Often only a subset of the peaks will have changes in resonance frequency upon binding to another molecule, allowing one to map onto the structure those residues directly involved in the interaction or involved in conformational changes as a result of the interaction. If the interacting

molecule is relatively large (protein or nucleic acid) the peak widths will also broaden due to the increased rotational correlation time of the complex. In some cases the peaks involved in the interaction may actually disappear from the NMR spectrum if the interacting molecule is in intermediate exchange on the NMR timescale (i.e., exchanging on

5 and off the polypeptide at a frequency that is similar to the resonance frequency of the monitored nuclei).

To facilitate the acquisition of NMR data on a large number of compounds (e.g., a library of synthetic or naturally-occurring small organic compounds), a sample changer may be employed. Using the sample changer, a larger number of samples, numbering 60 or  
10 more, may be run unattended. To facilitate processing of the NMR data, computer programs are used to transfer and automatically process the multiple one-dimensional NMR data.

In one embodiment, the invention provides a screening method for identifying small molecules capable of interacting with a polypeptide of the invention. In one example, the  
15 screening process begins with the generation or acquisition of either a T<sub>2</sub>-filtered or a diffusion-filtered one-dimensional proton spectrum of the compound or mixture of compounds. Means for generating T<sub>2</sub>-filtered or diffusion-filtered one-dimensional proton spectra are well known in the art (see, e.g., S. Meiboom and D. Gill, Rev. Sci. Instrum. 29:688(1958), S. J. Gibbs and C. S. Johnson, Jr. J. Main. Reson. 93:395-402 (1991) and A.  
20 S. Altieri, et al. J. Am. Chem. Soc. 117: 7566-7567 (1995)).

Following acquisition of the first spectrum for the molecules, the <sup>15</sup>N- or <sup>13</sup>C-labeled polypeptide is exposed to one or more molecules. Where more than one test compound is to be tested simultaneously, it is preferred to use a library of compounds such as a plurality of small molecules. Such molecules are typically dissolved in perdeuterated  
25 dimethylsulfoxide. The compounds in the library may be purchased from vendors or created according to desired needs.

Individual compounds may be selected inter alia on the basis of size and molecular diversity for maximizing the possibility of discovering compounds that interact with widely diverse binding sites of a polypeptide of the invention.

30 The NMR screening process of the present invention utilizes a range of test compound concentrations, e.g., from about 0.05 to about 1.0 mM. At those exemplary concentrations, compounds which are acidic or basic may significantly change the pH of buffered protein solutions. Chemical shifts are sensitive to pH changes as well as direct

binding interactions, and false-positive chemical shift changes, which are not the result of test compound binding but of changes in pH, may therefore be observed. It may therefore be necessary to ensure that the pH of the buffered solution does not change upon addition of the test compound.

5       Following exposure of the test compounds to a polypeptide (e.g., the target molecule for the experiment) a second one-dimensional T<sub>2</sub>- or diffusion-filtered spectrum is generated. For the T<sub>2</sub>-filtered approach, that second spectrum is generated in the same manner as set forth above. The first and second spectra are then compared to determine whether there are any differences between the two spectra. Differences in the one-dimensional T<sub>2</sub>-filtered spectra indicate that the compound is binding to, or otherwise interacting with, the target molecule. Those differences are determined using standard procedures well known in the art. For the diffusion-filtered method, the second spectrum is generated by looking at the spectral differences between low and high gradient strengths--thus selecting for those compounds whose diffusion rates are comparable to that observed  
10      in the absence of target molecule.  
15

To discover additional molecules that bind to the protein, molecules are selected for testing based on the structure/activity relationships from the initial screen and/or structural information on the initial leads when bound to the protein. By way of example, the initial screening may result in the identification of compounds, all of which contain an aromatic ring. The second round of screening would then use other aromatic molecules as the test compounds.  
20

In another embodiment, the methods of the invention utilize a process for detecting the binding of one ligand to a polypeptide in the presence of a second ligand. In accordance with this embodiment, a polypeptide is bound to the second ligand before exposing the polypeptide to the test compounds.  
25

For more information on NMR methods encompassed by the present invention, see also: U.S. Patent Nos. 5,668,734; 6,194,179; 6,162,627; 6,043,024; 5,817,474; 5,891,642; 5,989,827; 5,891,643; 6,077,682; WO 00/05414; WO 99/22019; Cavanagh, et al., Protein NMR Spectroscopy, Principles and Practice, 1996, Academic Press; Clore, et al., NMR of Proteins. In Topics in Molecular and Structural Biology, 1993, S. Neidle, Fuller, W., and Cohen, J.S., eds., Macmillan Press, Ltd., London; and Christendat et al., Nature Structural Biology 7: 903-909 (2000).  
30

*(c) Analysis of Proteins by X-ray Crystallography*

*(i) X-ray Structure Determination*

Exemplary methods for obtaining the three dimensional structure of the crystalline form of a molecule or complex are described herein and, in view of this specification, variations on these methods will be apparent to those skilled in the art (see Ducruix and 5 Geige 1992, IRL Press, Oxford, England).

A variety of methods involving x-ray crystallography are contemplated by the present invention. For example, the present invention contemplates producing a crystallized polypeptide of the invention, or a fragment thereof, by: (a) introducing into a host cell an expression vector comprising a nucleic acid encoding for a polypeptide of the 10 invention, or a fragment thereof; (b) culturing the host cell in a cell culture medium to express the polypeptide or fragment; (c) isolating the polypeptide or fragment from the cell culture; and (d) crystallizing the polypeptide or fragment thereof. Alternatively, the present invention contemplates determining the three dimensional structure of a crystallized polypeptide of the invention, or a fragment thereof, by: (a) crystallizing a polypeptide of the 15 invention, or a fragment thereof, such that the crystals will diffract x-rays to a resolution of 3.5 Å or better; and (b) analyzing the polypeptide or fragment by x-ray diffraction to determine the three-dimensional structure of the crystallized polypeptide.

X-ray crystallography techniques generally require that the protein molecules be available in the form of a crystal. Crystals may be grown from a solution containing a 20 purified polypeptide of the invention, or a fragment thereof (e.g., a stable domain), by a variety of conventional processes. These processes include, for example, batch, liquid, bridge, dialysis, vapour diffusion (e.g., hanging drop or sitting drop methods). (See for example, McPherson, 1982 John Wiley, New York; McPherson, 1990, Eur. J. Biochem. 189: 1-23; Webber. 1991, Adv. Protein Chem. 41:1-36).

25 In certain embodiments, native crystals of the invention may be grown by adding precipitants to the concentrated solution of the polypeptide. The precipitants are added at a concentration just below that necessary to precipitate the protein. Water may be removed by controlled evaporation to produce precipitating conditions, which are maintained until crystal growth ceases.

30 The formation of crystals is dependent on a number of different parameters, including pH, temperature, protein concentration, the nature of the solvent and precipitant, as well as the presence of added ions or ligands to the protein. In addition, the sequence of the polypeptide being crystallized will have a significant affect on the success of obtaining

crystals. Many routine crystallization experiments may be needed to screen all these parameters for the few combinations that might give crystal suitable for x-ray diffraction analysis (See, for example, Jancarik, J & Kim, S.H., *J. Appl. Cryst.* 1991 24: 409-411).

Crystallization robots may automate and speed up the work of reproducibly setting 5 up large number of crystallization experiments. Once some suitable set of conditions for growing the crystal are found, variations of the condition may be systematically screened in order to find the set of conditions which allows the growth of sufficiently large, single, well ordered crystals. In certain instances, a polypeptide of the invention is co-crystallized with a compound that stabilizes the polypeptide.

10 A number of methods are available to produce suitable radiation for x-ray diffraction. For example, x-ray beams may be produced by synchrotron rings where electrons (or positrons) are accelerated through an electromagnetic field while traveling at close to the speed of light. Because the admitted wavelength may also be controlled, synchrotrons may be used as a tunable x-ray source (Hendrickson WA., *Trends Biochem Sci* 2000 Dec; 25(12):637-43). For less conventional Laue diffraction studies, 15 polychromatic x-rays covering a broad wavelength window are used to observe many diffraction intensities simultaneously (Stoddard, B. L., *Curr. Opin. Struct Biol* 1998 Oct; 8(5):612-8). Neutrons may also be used for solving protein crystal structures (Gutberlet T, Heinemann U & Steiner M., *Acta Crystallogr D* 2001;57: 349-54).

20 Before data collection commences, a protein crystal may be frozen to protect it from radiation damage. A number of different cryo-protectants may be used to assist in freezing the crystal, such as methyl pentanediol (MPD), isopropanol, ethylene glycol, glycerol, formate, citrate, mineral oil, or a low-molecular-weight polyethylene glycol (PEG). The present invention contemplates a composition comprising a polypeptide of the invention 25 and a cryo-protectant. As an alternative to freezing the crystal, the crystal may also be used for diffraction experiments performed at temperatures above the freezing point of the solution. In these instances, the crystal may be protected from drying out by placing it in a narrow capillary of a suitable material (generally glass or quartz) with some of the crystal growth solution included in order to maintain vapour pressure.

30 X-ray diffraction results may be recorded by a number of ways known to one of skill in the art. Examples of area electronic detectors include charge coupled device detectors, multi-wire area detectors and phosphoimager detectors (Amemiya, Y, 1997. *Methods in Enzymology*, Vol. 276. Academic Press, San Diego, pp. 233-243; Westbrook, E. M.,

Naday, I. 1997. Methods in Enzymology, Vol. 276. Academic Press, San Diego, pp. 244-268; 1997. Kahn, R. & Fourme, R. Methods in Enzymology, Vol. 276. Academic Press, San Diego, pp. 268-286).

A suitable system for laboratory data collection might include a Bruker AXS

5 Proteum R system, equipped with a copper rotating anode source, Confocal Max-Flux™ optics and a SMART 6000 charge coupled device detector. Collection of x-ray diffraction patterns are well documented by those skilled in the art (See, for example, Ducruix and Geige, 1992, IRL Press, Oxford, England).

The theory behind diffraction by a crystal upon exposure to x-rays is well known.

10 Because phase information is not directly measured in the diffraction experiment, and is needed to reconstruct the electron density map, methods that can recover this missing information are required. One method of solving structures *ab initio* are the real / reciprocal space cycling techniques. Suitable real / reciprocal space cycling search programs include shake-and-bake (Weeks CM, DeTitta GT, Hauptman HA, Thuman P, Miller R Acta Crystallogr A 1994; V50: 210-20).

Other methods for deriving phases may also be needed. These techniques generally rely on the idea that if two or more measurements of the same reflection are made where strong, measurable, differences are attributable to the characteristics of a small subset of the atoms alone, then the contributions of other atoms can be, to a first approximation, ignored, 20 and positions of these atoms may be determined from the difference in scattering by one of the above techniques. Knowing the position and scattering characteristics of those atoms, one may calculate what phase the overall scattering must have had to produce the observed differences.

One version of this technique is isomorphous replacement technique, which requires 25 the introduction of new, well ordered, x-ray scatterers into the crystal. These additions are usually heavy metal atoms, (so that they make a significant difference in the diffraction pattern); and if the additions do not change the structure of the molecule or of the crystal cell, the resulting crystals should be isomorphous. Isomorphous replacement experiments are usually performed by diffusing different heavy-metal metals into the channels of a pre-existing protein crystal. Growing the crystal from protein that has been soaked in the heavy atom is also possible (Petsko, G.A., 1985. Methods in Enzymology, Vol. 114. Academic Press, Orlando, pp. 147-156). Alternatively, the heavy atom may also be reactive and attached covalently to exposed amino acid side chains (such as the sulfur atom of cysteine) 30

or it may be associated through non-covalent interactions. It is sometimes possible to replace endogenous light metals in metallo-proteins with heavier ones, e.g., zinc by mercury, or calcium by samarium (Petsko, G.A., 1985. Methods in Enzymology, Vol. 114. Academic Press, Orlando, pp. 147-156). Exemplary sources for such heavy compounds 5 include, without limitation, sodium bromide, sodium selenate, trimethyl lead acetate, mercuric chloride, methyl mercury acetate, platinum tetracyanide, platinum tetrachloride, nickel chloride, and europium chloride.

A second technique for generating differences in scattering involves the phenomenon of anomalous scattering. X-rays that cause the displacement of an electron in 10 an inner shell to a higher shell are subsequently rescattered, but there is a time lag that shows up as a phase delay. This phase delay is observed as a (generally quite small) difference in intensity between reflections known as Friedel mates that would be identical if no anomalous scattering were present. A second effect related to this phenomenon is that differences in the intensity of scattering of a given atom will vary in a wavelength 15 dependent manner, given rise to what are known as dispersive differences. In principle anomalous scattering occurs with all atoms, but the effect is strongest in heavy atoms, and may be maximized by using x-rays at a wavelength where the energy is equal to the difference in energy between shells. The technique therefore requires the incorporation of some heavy atom much as is needed for isomorphous replacement, although for anomalous 20 scattering a wider variety of atoms are suitable, including lighter metal atoms (copper, zinc, iron) in metallo-proteins. One method for preparing a protein for anomalous scattering involves replacing the methionine residues in whole or in part with selenium containing seleno-methionine. Soaks with halide salts such as bromides and other non-reactive ions 25 may also be effective (Dauter Z, Li M, Wlodawer A., Acta Crystallogr D 2001; 57: 239-49).

In another process, known as multiple anomalous scattering or MAD, two to four suitable wavelengths of data are collected. (Hendrickson, W.A. and Ogata, C.M. 1997 Methods in Enzymology 276, 494 – 523). Phasing by various combinations of single and 30 multiple isomorphous and anomalous scattering are possible too. For example, SIRAS (single isomorphous replacement with anomalous scattering) utilizes both the isomorphous and anomalous differences for one derivative to derive phases. More traditionally, several different heavy atoms are soaked into different crystals to get sufficient phase information from isomorphous differences while ignoring anomalous scattering, in the technique known

as multiple isomorphous replacement (MIR) (Petsko, G.A., 1985. Methods in Enzymology, Vol. 114. Academic Press, Orlando, pp. 147-156).

Additional restraints on the phases may be derived from density modification techniques. These techniques use either generally known features of electron density distribution or known facts about that particular crystal to improve the phases. For example, because protein regions of the crystal scatter more strongly than solvent regions, solvent flattening/flipping may be used to adjust phases to make solvent density a uniform flat value (Zhang, K. Y. J., Cowtan, K. and Main, P. Methods in Enzymology 277, 1997 Academic Press, Orlando pp 53-64). If more than one molecule of the protein is present in the asymmetric unit, the fact that the different molecules should be virtually identical may be exploited to further reduce phase error using non-crystallographic symmetry averaging (Villieux, F. M. D. and Read, R. J. Methods in Enzymology 277, 1997 Academic Press, Orlando pp18-52). Suitable programs for performing these processes include DM and other programs of the CCP4 suite (Collaborative Computational Project, Number 4. 1994. Acta Cryst. D50, 760-763) and CNX.

The unit cell dimensions, symmetry, vector amplitude and derived phase information can be used in a Fourier transform function to calculate the electron density in the unit cell, i.e., to generate an experimental electron density map. This may be accomplished using programs of the CNX or CCP4 packages. The resolution is measured in Ångstrom (Å) units, and is closely related to how far apart two objects need to be before they can be reliably distinguished. The smaller this number is, the higher the resolution and therefore the greater the amount of detail that can be seen. Preferably, crystals of the invention diffract x-rays to a resolution of better than about 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5 Å or better.

As used herein, the term "modeling" includes the quantitative and qualitative analysis of molecular structure and/or function based on atomic structural information and interaction models. The term "modeling" includes conventional numeric-based molecular dynamic and energy minimization models, interactive computer graphic models, modified molecular mechanics models, distance geometry and other structure-based constraint models.

Model building may be accomplished by either the crystallographer using a computer graphics program such as TURBO or O (Jones, TA. et al., Acta Crystallogr. A47, 100-119, 1991) or, under suitable circumstances, by using a fully automated model building

program, such as wARP (Anastassis Perrakis; Richard Morris & Victor S. Lamzin; *Nature Structural Biology*, May 1999 Volume 6 Number 5 pp 458 – 463) or MAID (Levitt, D. G., *Acta Crystallogr. D* 2001 V57: 1013-9). This structure may be used to calculate model-derived diffraction amplitudes and phases. The model-derived and experimental diffraction

5 amplitudes may be compared and the agreement between them can be described by a parameter referred to as R-factor. A high degree of correlation in the amplitudes corresponds to a low R-factor value, with 0.0 representing exact agreement and 0.59 representing a completely random structure. Because the R-factor may be lowered by introducing more free parameters into the model, an unbiased, cross-correlated version of  
10 the R-factor known as the R-free gives a more objective measure of model quality. For the calculation of this parameter a subset of reflections (generally around 10%) are set aside at the beginning of the refinement and not used as part of the refinement target. These reflections are then compared to those predicted by the model (Kleywegt GJ, Brunger AT, *Structure* 1996 Aug 15;4(8):897-904).

15 The model may be improved using computer programs that maximize the probability that the observed data was produced from the predicted model, while simultaneously optimizing the model geometry. For example, the CNX program may be used for model refinement, as can the XPLOR program (1992, *Nature* 355:472-475, G.N. Murshudov, A.A.Vagin and E.J.Dodson, (1997) *Acta Cryst. D* 53, 240-255). In order to  
20 maximize the convergence radius of refinement, simulated annealing refinement using torsion angle dynamics may be employed in order to reduce the degrees of freedom of motion of the model (Adams PD, Pannu NS, Read RJ, Brunger AT., *Proc Natl Acad Sci U S A* 1997 May 13;94(10):5018-23). Where experimental phase information is available (e.g. where MAD data was collected) Hendrickson-Lattman phase probability targets may  
25 be employed. Isotropic or anisotropic domain, group or individual temperature factor refinement, may be used to model variance of the atomic position from its mean. Well defined peaks of electron density not attributable to protein atoms are generally modeled as water molecules. Water molecules may be found by manual inspection of electron density maps, or with automatic water picking routines. Additional small molecules, including  
30 ions, cofactors, buffer molecules or substrates may be included in the model if sufficiently unambiguous electron density is observed in a map.

In general, the R-free is rarely as low as 0.15 and may be as high as 0.35 or greater for a reasonably well-determined protein structure. The residual difference is a

consequence of approximations in the model (inadequate modeling of residual structure in the solvent, modeling atoms as isotropic Gaussian spheres, assuming all molecules are identical rather than having a set of discrete conformers, etc.) and errors in the data (Lattman EE., Proteins 1996; 25: i-ii). In refined structures at high resolution, there are  
5 usually no major errors in the orientation of individual residues, and the estimated errors in atomic positions are usually around 0.1 - 0.2 up to 0.3 Å.

The three dimensional structure of a new crystal may be modeled using molecular replacement. The term "molecular replacement" refers to a method that involves generating a preliminary model of a molecule or complex whose structure coordinates are unknown,  
10 by orienting and positioning a molecule whose structure coordinates are known within the unit cell of the unknown crystal, so as best to account for the observed diffraction pattern of the unknown crystal. Phases may then be calculated from this model and combined with the observed amplitudes to give an approximate Fourier synthesis of the structure whose coordinates are unknown. This, in turn, can be subject to any of the several forms of  
15 refinement to provide a final, accurate structure of the unknown crystal. Lattman, E., "Use of the Rotation and Translation Functions", in Methods in Enzymology, 115, pp. 55-77 (1985); M. G. Rossmann, ed., "The Molecular Replacement Method", Int. Sci. Rev. Ser., No. 13, Gordon & Breach, New York, (1972).

Commonly used computer software packages for molecular replacement are CNX,  
20 X-PLOR (Brünger 1992, Nature 355: 472-475), AMoRE (Navaza, 1994, Acta Crystallogr. A50:157-163), the CCP4 package, the MERLOT package (P.M.D. Fitzgerald, J. Appl. Cryst., Vol. 21, pp. 273-278, 1988) and XTALVIEW (McCree et al (1992) J. Mol. Graphics 10: 44-46). The quality of the model may be analyzed using a program such as PROCHECK or 3D-Profiler (Laskowski et al 1993 J. Appl. Cryst. 26:283-291; Luthy R. et  
25 al, Nature 356: 83-85, 1992; and Bowie, J.U. et al, Science 253: 164-170, 1991).

Homology modeling (also known as comparative modeling or knowledge-based modeling) methods may also be used to develop a three dimensional model from a polypeptide sequence based on the structures of known proteins. The method utilizes a computer model of a known protein, a computer representation of the amino acid sequence  
30 of the polypeptide with an unknown structure, and standard computer representations of the structures of amino acids. This method is well known to those skilled in the art (Greer, 1985, Science 228, 1055; Bundell et al 1988, Eur. J. Biochem. 172, 513; Knighton et al., 1992, Science 258:130-135, <http://biochem.vt.edu/courses/-modeling/homology.htm>).

Computer programs that can be used in homology modeling are QUANTA and the Homology module in the Insight II modeling package distributed by Molecular Simulations Inc, or MODELLER (Rockefeller University, [www.iucr.ac.uk/sinris-top/logical/prg-modeller.html](http://www.iucr.ac.uk/sinris-top/logical/prg-modeller.html)).

5 Once a homology model has been generated it is analyzed to determine its correctness. A computer program available to assist in this analysis is the Protein Health module in QUANTA which provides a variety of tests. Other programs that provide structure analysis along with output include PROCHECK and 3D-Profiler (Luthy R. et al, Nature 356: 83-85, 1992; and Bowie, J.U. et al, Science 253: 164-170, 1991). Once any  
10 irregularities have been resolved, the entire structure may be further refined.

Other molecular modeling techniques may also be employed in accordance with this invention. See, e.g., Cohen, N. C. *et al*, J. Med. Chem., 33, pp. 883-894 (1990). See also, Navix, M. A. and M. A. Marko, Current Opinions in Structural Biology, 2, pp. 202-210 (1992).

15 Under suitable circumstances, the entire process of solving a crystal structure may be accomplished in an automated fashion by a system such as ELVES (<http://ucxray.berkeley.edu/~jamesh/elves/index.html>) with little or no user intervention.

(ii) *X-ray Structure*

The present invention provides methods for determining some or all of the structural  
20 coordinates for amino acids of a polypeptide of the invention, or a complex thereof.

In another aspect, the present invention provides methods for identifying a druggable region of a polypeptide of the invention. For example, one such method includes: (a) obtaining crystals of a polypeptide of the invention or a fragment thereof such that the three dimensional structure of the crystallized protein can be determined to a  
25 resolution of 3.5 Å or better; (b) determining the three dimensional structure of the crystallized polypeptide or fragment using x-ray diffraction; and (c) identifying a druggable region of a polypeptide of the invention based on the three-dimensional structure of the polypeptide or fragment.

A three dimensional structure of a molecule or complex may be described by the set  
30 of atoms that best predict the observed diffraction data (that is, which possesses a minimal R value). Files may be created for the structure that defines each atom by its chemical identity, spatial coordinates in three dimensions, root mean squared deviation from the mean observed position and fractional occupancy of the observed position.

Those of skill in the art understand that a set of structure coordinates for an protein, complex or a portion thereof, is a relative set of points that define a shape in three dimensions. Thus, it is possible that an entirely different set of coordinates could define a similar or identical shape. Moreover, slight variations in the individual coordinates may 5 have little affect on overall shape. Such variations in coordinates may be generated because of mathematical manipulations of the structure coordinates. For example, structure coordinates could be manipulated by crystallographic permutations of the structure coordinates, fractionalization of the structure coordinates, integer additions or subtractions to sets of the structure coordinates, inversion of the structure coordinates or any 10 combination of the above. Alternatively, modifications in the crystal structure due to mutations, additions, substitutions, and/or deletions of amino acids, or other changes in any of the components that make up the crystal, could also yield variations in structure coordinates. Such slight variations in the individual coordinates will have little affect on overall shape. If such variations are within an acceptable standard error as compared to the 15 original coordinates, the resulting three-dimensional shape is considered to be structurally equivalent. It should be noted that slight variations in individual structure coordinates of a polypeptide of the invention or a complex thereof would not be expected to significantly alter the nature of modulators that could associate with a druggable region thereof. Thus, for example, a modulator that bound to the active site of a polypeptide of the invention 20 would also be expected to bind to or interfere with another active site whose structure coordinates define a shape that falls within the acceptable error.

A crystal structure of the present invention may be used to make a structural or computer model of the polypeptide, complex or portion thereof. A model may represent the secondary, tertiary and/or quaternary structure of the polypeptide, complex or portion. The 25 configurations of points in space derived from structure coordinates according to the invention can be visualized as, for example, a holographic image, a stereodiagram, a model or a computer-displayed image, and the invention thus includes such images, diagrams or models.

*(iii) Structural Equivalents*

30 Various computational analyses can be used to determine whether a molecule or the active site portion thereof is structurally equivalent with respect to its three-dimensional structure, to all or part of a structure of a polypeptide of the invention or a portion thereof.

For the purpose of this invention, any molecule or complex or portion thereof, that has a root mean square deviation of conserved residue backbone atoms (N, Ca, C, O) of less than about 1.75 Å, when superimposed on the relevant backbone atoms described by the reference structure coordinates of a polypeptide of the invention, is considered 5 "structurally equivalent" to the reference molecule. That is to say, the crystal structures of those portions of the two molecules are substantially identical, within acceptable error. Alternatively, the root mean square deviation may be less than about 1.50, 1.40, 1.25, 1.0, 0.75, 0.5 or 0.35 Å.

The term "root mean square deviation" is understood in the art and means the square 10 root of the arithmetic mean of the squares of the deviations. It is a way to express the deviation or variation from a trend or object.

In another aspect, the present invention provides a scalable three-dimensional configuration of points, at least a portion of said points, and preferably all of said points, derived from structural coordinates of at least a portion of a polypeptide of the invention 15 and having a root mean square deviation from the structure coordinates of the polypeptide of the invention of less than 1.50, 1.40, 1.25, 1.0, 0.75, 0.5 or 0.35 Å. In certain embodiments, the portion of a polypeptide of the invention is 25%, 33%, 50%, 66%, 75%, 85%, 90% or 95% or more of the amino acid residues contained in the polypeptide.

In another aspect, the present invention provides a molecule or complex including a 20 druggable region of a polypeptide of the invention, the druggable region being defined by a set of points having a root mean square deviation of less than about 1.75 Å from the structural coordinates for points representing (a) the backbone atoms of the amino acids contained in a druggable region of a polypeptide of the invention, (b) the side chain atoms (and optionally the Ca atoms) of the amino acids contained in such druggable region, or 25 (c) all the atoms of the amino acids contained in such druggable region. In certain embodiments, only a portion of the amino acids of a druggable region may be included in the set of points, such as 25%, 33%, 50%, 66%, 75%, 85%, 90% or 95% or more of the amino acid residues contained in the druggable region. In certain embodiments, the root mean square deviation may be less than 1.50, 1.40, 1.25, 1.0, 0.75, 0.5, or 0.35 Å. In still 30 other embodiments, instead of a druggable region, a stable domain, fragment or structural motif is used in place of a druggable region.

*(iv) Machine Displays and Machine Readable Storage Media*

The invention provides a machine-readable storage medium including a data storage material encoded with machine readable data which, when using a machine programmed with instructions for using said data, displays a graphical three-dimensional representation of any of the molecules or complexes, or portions thereof, of this invention. In another 5 embodiment, the graphical three-dimensional representation of such molecule, complex or portion thereof includes the root mean square deviation of certain atoms of such molecule by a specified amount, such as the backbone atoms by less than 0.8 Å. In another embodiment, a structural equivalent of such molecule, complex, or portion thereof, may be displayed. In another embodiment, the portion may include a druggable region of the 10 polypeptide of the invention.

According to one embodiment, the invention provides a computer for determining at least a portion of the structure coordinates corresponding to x-ray diffraction data obtained from a molecule or complex, wherein said computer includes: (a) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, 15 wherein said data comprises at least a portion of the structural coordinates of a polypeptide of the invention; (b) a machine-readable data storage medium comprising a data storage material encoded with machine-readable data, wherein said data comprises x-ray diffraction data from said molecule or complex; (c) a working memory for storing instructions for processing said machine-readable data of (a) and (b); (d) a central-processing unit coupled 20 to said working memory and to said machine-readable data storage medium of (a) and (b) for performing a Fourier transform of the machine readable data of (a) and for processing said machine readable data of (b) into structure coordinates; and (e) a display coupled to said central-processing unit for displaying said structure coordinates of said molecule or complex. In certain embodiments, the structural coordinates displayed are structurally 25 equivalent to the structural coordinates of a polypeptide of the invention.

In an alternative embodiment, the machine-readable data storage medium includes a data storage material encoded with a first set of machine readable data which includes the Fourier transform of the structure coordinates of a polypeptide of the invention or a portion thereof, and which, when using a machine programmed with instructions for using said 30 data, can be combined with a second set of machine readable data including the x-ray diffraction pattern of a molecule or complex to determine at least a portion of the structure coordinates corresponding to the second set of machine readable data.

For example, a system for reading a data storage medium may include a computer including a central processing unit ("CPU"), a working memory which may be, e.g., RAM (random access memory) or "core" memory, mass storage memory (such as one or more disk drives or CD-ROM drives), one or more display devices (e.g., cathode-ray tube 5 ("CRT") displays, light emitting diode ("LED") displays, liquid crystal displays ("LCDs"), electroluminescent displays, vacuum fluorescent displays, field emission displays ("FEDs"), plasma displays, projection panels, etc.), one or more user input devices (e.g., keyboards, microphones, mice, touch screens, etc.), one or more input lines, and one or more output lines, all of which are interconnected by a conventional bidirectional system 10 bus. The system may be a stand-alone computer, or may be networked (e.g., through local area networks, wide area networks, intranets, extranets, or the internet) to other systems (e.g., computers, hosts, servers, etc.). The system may also include additional computer controlled devices such as consumer electronics and appliances.

Input hardware may be coupled to the computer by input lines and may be 15 implemented in a variety of ways. Machine-readable data of this invention may be inputted via the use of a modem or modems connected by a telephone line or dedicated data line. Alternatively or additionally, the input hardware may include CD-ROM drives or disk drives. In conjunction with a display terminal, a keyboard may also be used as an input device.

Output hardware may be coupled to the computer by output lines and may similarly 20 be implemented by conventional devices. By way of example, the output hardware may include a display device for displaying a graphical representation of an active site of this invention using a program such as QUANTA as described herein. Output hardware might also include a printer, so that hard copy output may be produced, or a disk drive, to store 25 system output for later use.

In operation, a CPU coordinates the use of the various input and output devices, coordinates data accesses from mass storage devices, accesses to and from working memory, and determines the sequence of data processing steps. A number of programs may be used to process the machine-readable data of this invention. Such programs are 30 discussed in reference to the computational methods of drug discovery as described herein. References to components of the hardware system are included as appropriate throughout the following description of the data storage medium.

Machine-readable storage devices useful in the present invention include, but are not limited to, magnetic devices, electrical devices, optical devices, and combinations thereof. Examples of such data storage devices include, but are not limited to, hard disk devices, CD devices, digital video disk devices, floppy disk devices, removable hard disk devices, magneto-optic disk devices, magnetic tape devices, flash memory devices, bubble memory devices, holographic storage devices, and any other mass storage peripheral device. It should be understood that these storage devices include necessary hardware (e.g., drives, controllers, power supplies, etc.) as well as any necessary media (e.g., disks, flash cards, etc.) to enable the storage of data.

10 In one embodiment, the present invention contemplates a computer readable storage medium comprising structural data, wherein the data include the identity and three-dimensional coordinates of a polypeptide of the invention or portion thereof. In another aspect, the present invention contemplates a database comprising the identity and three-dimensional coordinates of a polypeptide of the invention or a portion thereof.

15 Alternatively, the present invention contemplates a database comprising a portion or all of the atomic coordinates of a polypeptide of the invention or portion thereof.

(v) *Structurally Similar Molecules and Complexes*

Structural coordinates for a polypeptide of the invention can be used to aid in obtaining structural information about another molecule or complex. This method of the invention allows determination of at least a portion of the three-dimensional structure of molecules or molecular complexes which contain one or more structural features that are similar to structural features of a polypeptide of the invention. Similar structural features can include, for example, regions of amino acid identity, conserved active site or binding site motifs, and similarly arranged secondary structural elements (e.g.,  $\alpha$  helices and  $\beta$  sheets). Many of the methods described above for determining the structure of a polypeptide of the invention may be used for this purpose as well.

For the present invention, a "structural homolog" is a polypeptide that contains one or more amino acid substitutions, deletions, additions, or rearrangements with respect to the amino acid sequence of SEQ ID NO: 4 or other polypeptide of the invention, but that, when folded into its native conformation, exhibits or is reasonably expected to exhibit at least a portion of the tertiary (three-dimensional) structure of the polypeptide encoded by SEQ ID NO: 4 or such other polypeptide of the invention. For example, structurally homologous molecules can contain deletions or additions of one or more contiguous or noncontiguous

amino acids, such as a loop or a domain. Structurally homologous molecules also include modified polypeptide molecules that have been chemically or enzymatically derivatized at one or more constituent amino acids, including side chain modifications, backbone modifications, and N- and C-terminal modifications including acetylation, hydroxylation, 5 methylation, amidation, and the attachment of carbohydrate or lipid moieties, cofactors, and the like.

By using molecular replacement, all or part of the structure coordinates of a polypeptide of the invention can be used to determine the structure of a crystallized molecule or complex whose structure is unknown more quickly and efficiently than 10 attempting to determine such information *ab initio*. For example, in one embodiment this invention provides a method of utilizing molecular replacement to obtain structural information about a molecule or complex whose structure is unknown including: (a) crystallizing the molecule or complex of unknown structure; (b) generating an x-ray diffraction pattern from said crystallized molecule or complex; and (c) applying at least a 15 portion of the structure coordinates for a polypeptide of the invention to the x-ray diffraction pattern to generate a three-dimensional electron density map of the molecule or complex whose structure is unknown.

In another aspect, the present invention provides a method for generating a preliminary model of a molecule or complex whose structure coordinates are unknown, by 20 orienting and positioning the relevant portion of a polypeptide of the invention within the unit cell of the crystal of the unknown molecule or complex so as best to account for the observed x-ray diffraction pattern of the crystal of the molecule or complex whose structure is unknown.

Structural information about a portion of any crystallized molecule or complex that 25 is sufficiently structurally similar to a portion of a polypeptide of the invention may be resolved by this method. In addition to a molecule that shares one or more structural features with a polypeptide of the invention, a molecule that has similar bioactivity, such as the same catalytic activity, substrate specificity or ligand binding activity as a polypeptide of the invention, may also be sufficiently structurally similar to a polypeptide of the 30 invention to permit use of the structure coordinates for a polypeptide of the invention to solve its crystal structure.

In another aspect, the method of molecular replacement is utilized to obtain structural information about a complex containing a polypeptide of the invention, such as a

complex between a modulator and a polypeptide of the invention (or a domain, fragment, ortholog, homolog etc. thereof). In certain instances, the complex includes a polypeptide of the invention (or a domain, fragment, ortholog, homolog etc. thereof) co-complexed with a modulator. For example, in one embodiment, the present invention contemplates a method

- 5 for making a crystallized complex comprising a polypeptide of the invention, or a fragment thereof, and a compound having a molecular weight of less than 5 kDa, the method comprising: (a) crystallizing a polypeptide of the invention such that the crystals will diffract x-rays to a resolution of 3.5 Å or better; and (b) soaking the crystal in a solution comprising the compound having a molecular weight of less than 5 kDa, thereby producing
- 10 a crystallized complex comprising the polypeptide and the compound.

Using homology modeling, a computer model of a structural homolog or other polypeptide can be built or refined without crystallizing the molecule. For example, in another aspect, the present invention provides a computer-assisted method for homology modeling a structural homolog of a polypeptide of the invention including: aligning the  
15 amino acid sequence of a known or suspected structural homolog with the amino acid sequence of a polypeptide of the invention and incorporating the sequence of the homolog into a model of a polypeptide of the invention derived from atomic structure coordinates to yield a preliminary model of the homolog; subjecting the preliminary model to energy minimization to yield an energy minimized model; remodeling regions of the energy  
20 minimized model where stereochemistry restraints are violated to yield a final model of the homolog.

In another embodiment, the present invention contemplates a method for determining the crystal structure of a homolog of a polypeptide having SEQ ID NO: 2 or SEQ ID NO: 4, or equivalent thereof, the method comprising: (a) providing the three  
25 dimensional structure of a crystallized polypeptide having SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment thereof; (b) obtaining crystals of a homologous polypeptide comprising an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 such that the three dimensional structure of the crystallized homologous polypeptide may be determined to a resolution of 3.5 Å or better;  
30 and (c) determining the three dimensional structure of the crystallized homologous polypeptide by x-ray crystallography based on the atomic coordinates of the three dimensional structure provided in step (a). In certain instances of the foregoing method, the atomic coordinates for the homologous polypeptide have a root mean square deviation from

the backbone atoms of the polypeptide having SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment thereof, of not more than 1.5 Å for all backbone atoms shared in common with the homologous polypeptide and the polypeptide having SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment thereof.

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*(vi) NMR Analysis Using X-ray Structural Data*

In another aspect, the structural coordinates of a known crystal structure may be applied to nuclear magnetic resonance data to determine the three dimensional structures of polypeptides with uncharacterized or incompletely characterized structure. (See for example, Wuthrich, 1986, John Wiley and Sons, New York: 176-199; Pflugrath et al., 1986,

10 J. Molecular Biology 189: 383-386; Kline et al., 1986 J. Molecular Biology 189:377-382).

While the secondary structure of a polypeptide may often be determined by NMR data, the spatial connections between individual pieces of secondary structure are not as readily determined. The structural coordinates of a polypeptide defined by x-ray crystallography can guide the NMR spectroscopist to an understanding of the spatial interactions between 15 secondary structural elements in a polypeptide of related structure. Information on spatial interactions between secondary structural elements can greatly simplify NOE data from two-dimensional NMR experiments. In addition, applying the structural coordinates after the determination of secondary structure by NMR techniques simplifies the assignment of NOE's relating to particular amino acids in the polypeptide sequence.

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In an embodiment, the invention relates to a method of determining three dimensional structures of polypeptides with unknown structures, by applying the structural coordinates of a crystal of the present invention to nuclear magnetic resonance data of the unknown structure. This method comprises the steps of: (a) determining the secondary structure of an unknown structure using NMR data; and (b) simplifying the assignment of 25 through-space interactions of amino acids. The term "through-space interactions" defines the orientation of the secondary structural elements in the three dimensional structure and the distances between amino acids from different portions of the amino acid sequence. The term "assignment" defines a method of analyzing NMR data and identifying which amino acids give rise to signals in the NMR spectrum.

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For all of this section on x-ray crystallography, see also Brooks et al. (1983) *J Comput Chem* 4:187-217; Weiner et al (1981) *J. Comput. Chem.* 106: 765; Eisenfield et al. (1991) *Am J Physiol* 261:C376-386; Lybrand (1991) *J Pharm Belg* 46:49-54; Froimowitz (1990) *Biotechniques* 8:640-644; Burbam et al. (1990) *Proteins* 7:99-111; Pedersen (1985)

*Environ Health Perspect* 61:185-190; and Kini et al. (1991) *J Biomol Struct Dyn* 9:475-488; Ryckaert et al. (1977) *J Comput Phys* 23:327; Van Gunsteren et al. (1977) *Mol Phys* 34:1311; Anderson (1983) *J Comput Phys* 52:24; J. Mol. Biol. 48: 442-453, 1970; Dayhoff et al., *Meth. Enzymol.* 91: 524-545, 1983; Henikoff and Henikoff, *Proc. Nat. Acad. Sci. USA* 89: 10915-10919, 1992; J. Mol. Biol. 233: 716-738, 1993; *Methods in Enzymology*, Volume 276, Macromolecular crystallography, Part A, ISBN 0-12-182177-3 and Volume 277, Macromolecular crystallography, Part B, ISBN 0-12-182178-1, Eds. Charles W. Carter, Jr. and Robert M. Sweet (1997), Academic Press, San Diego; Pfuetzner, et al., *J. Biol. Chem.* 272: 430-434 (1997).

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#### 6. Interacting Proteins

The present invention also provides methods for isolating specific protein interactors of a polypeptide of the invention, and complexes comprising a polypeptide of the invention and one or more interacting proteins. In one aspect, the present invention contemplates an isolated protein complex comprising a polypeptide of the invention and at least one protein that interacts with the polypeptide of the invention. The protein may be naturally-occurring. The interacting protein may be of *P. aeruginosa* origin. Alternatively, the interacting protein may be of mammalian origin or human origin. Either the polypeptide of the invention or the interacting protein or both may be a fusion protein.

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The present invention contemplates a method for identifying a protein capable of interacting with a polypeptide of the invention or a fragment thereof, the method comprising: (a) exposing a sample to a solid substrate coupled to a polypeptide of the invention or a fragment thereof under conditions which promote protein-protein interactions; (b) washing the solid substrate so as to remove any polypeptides interacting non-specifically with the polypeptide or fragment; (c) eluting the polypeptides which specifically interact with the polypeptide or fragment; and (d) identifying the interacting protein. The sample may be an extract of *P. aeruginosa*, a mammalian cell extract, a human cell extract, a purified protein (or a fragment thereof), or a mixture of purified proteins (or fragments thereof). The interacting protein may be identified by a number of methods, including mass spectrometry or protein sequencing.

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In another aspect, the present invention contemplates a method for identifying a protein capable of interacting with a polypeptide of present invention or a fragment thereof, the method comprising: (a) subjecting a sample to protein-affinity chromatography on

multiple columns, the columns having a polypeptide of the invention or a fragment thereof coupled to the column matrix in varying concentrations, and eluting bound components of the extract from the columns; (b) separating the components to isolate a polypeptide capable of interacting with the polypeptide or fragment; and (c) analyzing the interacting protein by mass spectrometry to identify the interacting protein. In certain instances, the foregoing method will use polyacrylamide gel electrophoresis without SDS.

In another aspect, the present invention contemplates a method for identifying a protein capable of interacting with a polypeptide of the invention, the method comprising: (a) subjecting a cellular extract or extracellular fluid to protein-affinity chromatography on multiple columns, the columns having a polypeptide of the invention or a fragment thereof coupled to the column matrix in varying concentrations, and eluting bound components of the extract from the columns; (b) gel-separating the components to isolate an interacting protein; wherein the interacting protein is observed to vary in amount in direct relation to the concentration of coupled polypeptide or fragment; (c) digesting the interacting protein to give corresponding peptides; (d) analyzing the peptides by MALDI-TOF mass spectrometry or post source decay to determine the peptide masses; and (d) performing correlative database searches with the peptide, or peptide fragment, masses, whereby the interacting protein is identified based on the masses of the peptides or peptide fragments. The foregoing method may include the further step of including the identities of any interacting proteins into a relational database.

In another aspect, the invention further contemplates a method for identifying modulators of a protein complex, the method comprising: (a) contacting a protein complex comprising a polypeptide of the invention and an interacting protein with one or more test compounds; and (b) determining the effect of the test compound on (i) the activity of the protein complex, (ii) the amount of the protein complex, (iii) the stability of the protein complex, (iv) the conformation of the protein complex, (v) the activity of at least one polypeptide included in the protein complex, (vi) the conformation of at least one polypeptide included in the protein complex, (vii) the intracellular localization of the protein complex or a component thereof, (viii) the transcription level of a gene dependent on the complex, and/or (ix) the level of second messenger levels in a cell; thereby identifying modulators of the protein complex. The foregoing method may be carried out *in vitro* or *in vivo* as appropriate.

Typically, it will be desirable to immobilize a polypeptide of the invention to facilitate separation of complexes comprising a polypeptide of the invention from uncomplexed forms of the interacting proteins, as well as to accommodate automation of the assay. The polypeptide of the invention, or ligand, may be immobilized onto a solid support (e.g., column matrix, microtiter plate, slide, etc.). In certain embodiments, the ligand may be purified. In certain instances, a fusion protein may be provided which adds a domain that permits the ligand to be bound to a support.

In various *in vitro* embodiments, the set of proteins engaged in a protein-protein interaction comprises a cell extract, a clarified cell extract, or a reconstituted protein mixture of at least semi-purified proteins. By semi-purified, it is meant that the proteins utilized in the reconstituted mixture have been previously separated from other cellular or viral proteins. For instance, in contrast to cell lysates, the proteins involved in a protein-protein interaction are present in the mixture to at least about 50% purity relative to all other proteins in the mixture, and more preferably are present in greater, even 90-95%, purity. In certain embodiments of the subject method, the reconstituted protein mixture is derived by mixing highly purified proteins such that the reconstituted mixture substantially lacks other proteins (such as of cellular or viral origin) which might interfere with or otherwise alter the ability to measure activity resulting from the given protein-protein interaction.

Complex formation involving a polypeptide of the invention and another component polypeptide or a substrate polypeptide, may be detected by a variety of techniques. For instance, modulation in the formation of complexes can be quantitated using, for example, detectably labeled proteins (e.g. radiolabeled, fluorescently labeled, or enzymatically labeled), by immunoassay, or by chromatographic detection.

The present invention also provides assays for identifying molecules which are modulators of a protein-protein interaction involving a polypeptide of the invention, or are a modulator of the role of the complex comprising a polypeptide of the invention in the infectivity or pathogenicity of *P. aeruginosa*. In one embodiment, the assay detects agents which inhibit formation or stabilization of a protein complex comprising a polypeptide of the invention and one or more additional proteins. In another embodiment, the assay detects agents which modulate the intrinsic biological activity of a protein complex comprising a polypeptide of the invention, such as an enzymatic activity, binding to other cellular components, cellular compartmentalization, signal transduction, and the like. Such

modulators may be used, for example, in the treatment of *P. aeruginosa* related diseases or disorders. In certain embodiments, the compound is a mechanism based inhibitor which chemically alters one member of a protein-protein interaction involving a polypeptide of the invention and which is a specific inhibitor of that member, e.g. has an inhibition constant 5 about 10-fold, 100-fold, or 1000-fold different compared to homologous proteins.

In one embodiment, proteins that interact with a polypeptide of the invention may be isolated using immunoprecipitation. A polypeptide of the invention may be expressed in *P. aeruginosa*, or in a heterologous system. The cells expressing a polypeptide of the invention are then lysed under conditions which maintain protein-protein interactions, and 10 complexes comprising a polypeptide of the invention are isolated. For example, a polypeptide of the invention may be expressed in mammalian cells, including human cells, in order to identify mammalian proteins that interact with a polypeptide of the invention and therefore may play a role in *P. aeruginosa* infectivity or proliferation. In one embodiment, a polypeptide of the invention is expressed in the cell type for which it is 15 desirable to find interacting proteins. For example, a polypeptide of the invention may be expressed in *P. aeruginosa* in order to find *P. aeruginosa* derived interacting proteins.

In an alternative embodiment, a polypeptide of the invention is expressed and purified and then mixed with a potential interacting protein or mixture of proteins to identify complex formation. The potential interacting protein may be a single purified or 20 semi-purified protein, or a mixture of proteins, including a mixture of purified or semi-purified proteins, a cell lysate, a clarified cell lysate, a semi-purified cell lysate, etc.

In certain embodiments, it may be desirable to use a tagged version of a polypeptide of the invention in order to facilitate isolation of complexes from the reaction mixture. Suitable tags for immunoprecipitation experiments include HA, myc, FLAG, HIS, GST, 25 protein A, protein G, etc. Immunoprecipitation from a cell lysate or other protein mixture may be carried out using an antibody specific for a polypeptide of the invention or using an antibody which recognizes a tag to which a polypeptide of the invention is fused (e.g., anti-HA, anti-myc, anti-FLAG, etc.). Antibodies specific for a variety of tags are known to the skilled artisan and are commercially available from a number of sources. In the case where 30 a polypeptide of the invention is fused to a His, GST, or protein A/G tag, immunoprecipitation may be carried out using the appropriate affinity resin (e.g., beads functionalized with Ni, glutathione, Fc region of IgG, etc.). Test compounds which modulate a protein-protein interaction involving a polypeptide of the invention may be

identified by carrying out the immunoprecipitation reaction in the presence and absence of the test agent and comparing the level and/or activity of the protein complex between the two reactions.

In another embodiment, proteins that interact with a polypeptide of the invention 5 may be identified using affinity chromatography. Some examples of such chromatography are described in USSN 09/727,812, filed November 30, 2000, and the PCT Application filed November 30, 2001 and entitled "Methods for Systematic Identification of Protein-Protein Interactions and other Properties", which claims priority to such U.S. application.

In one aspect, for affinity chromatography using a solid support, a polypeptide of 10 the invention or a fragment thereof may be attached by a variety of means known to those of skill in the art. For example, the polypeptide may be coupled directly (through a covalent linkage) to commercially available pre-activated resins as described in Formosa et al., Methods in Enzymology 1991, 208, 24-45; Septa et al, J. Biol. Chem. 1985, 260, 10353-60; Archambault et al., Proc. Natl. Acad. Sci. USA 1997, 94, 14300-5. 15 Alternatively, the polypeptide may be tethered to the solid support through high affinity binding interactions. If the polypeptide is expressed fused to a tag, such as GST, the fusion tag can be used to anchor the polypeptide to the matrix support, for example Sepharose beads containing immobilized glutathione. Solid supports that take advantage of these tags are commercially available.

20 In another aspect, the support to which a polypeptide may be immobilized is a soluble support, which may facilitate certain steps performed in the methods of the present invention. For example, the soluble support may be soluble in the conditions employed to create a binding interaction between a target and the polypeptide, and then used under conditions in which it is a solid for elution of the proteins or other biological materials that bind to a polypeptide. 25

The concentration of the coupled polypeptide may have an affect on the sensitivity 30 of the method. In certain embodiments, to detect interactions most efficiently, the concentration of the polypeptide bound to the matrix should be at least 10-fold higher than the  $K_d$  of the interaction. Thus, the concentration of the polypeptide bound to the matrix should be highest for the detection of the weakest protein-protein interactions. However, if the concentration of the immobilized polypeptide is not as high as may be ideal, it may still be possible to observe protein-protein interactions of interest by, for example, increasing the concentration of the polypeptide or other moiety that interacts with the coupled

polypeptide. The level of detection will of course vary with each different polypeptide, interactor, conditions of the assay, etc. In certain instances, the interacting protein binds to the polypeptide with a  $K_d$  of about  $10^{-5}$  M to about  $10^{-8}$  M or  $10^{-10}$  M.

In another aspect, the coupling may be done at various ratios of the polypeptide to 5 the resin. An upper limit of the protein : resin ratio may be determined by the isoelectric point and the ionic nature of the protein, although it may be possible to achieve higher polypeptide concentrations by use of various methods.

In certain embodiments, several concentrations of the polypeptide immobilized on a solid or soluble support may be used. One advantage of using multiple concentrations, 10 although not a requirement, is that one may be able to obtain an estimate for the strength of the protein-protein interaction that is observed in the affinity chromatography experiment. Another advantage of using multiple concentrations is that a binding curve which has the proper shape may indicate that the interaction that is observed is biologically important rather than a spurious interaction with denatured protein.

15 In one example of such an embodiment, a series of columns may be prepared with varying concentrations of polypeptide (mg polypeptide/ml resin volume). The number of columns employed may be between 2 to 8, 10, 12, 15, 25 or more, each with a different concentration of attached polypeptide. Larger numbers of columns may be used if appropriate for the polypeptide being examined, and multiple columns may be used with 20 the same concentration as any methods may require. In certain embodiments, 4 to 6 columns are prepared with varying concentrations of polypeptide. In another aspect of this embodiment, two control columns may be prepared: one that contains no polypeptide and a second that contains the highest concentration of polypeptide but is not treated with extract. After elution of the columns and separation of the eluent components (by one of the 25 methods described below), it may be possible to distinguish the interacting proteins (if any) from the non-specific bound proteins as follows. The concentration of the interacting proteins, as determined by the intensity of the band on the gel, will increase proportionally to the increase in polypeptide concentration but will be missing from the second control column. This allows for the identification of unknown interacting proteins.

30 The method of the invention may be used for small-scale analysis. A variety of column sizes, types, and geometries may be used. In addition, other vessel shapes and sizes having a smaller scale than is usually found in laboratory experiments may be used as well, including a plurality of wells in a plate. For high throughput analysis, it is advantageous to

use small volumes, from about 20, 30, 50, 80 or 100 µl. Larger or small volumes may be used, as necessary, and it may be possible to achieve high throughput analysis using them. The entire affinity chromatography procedure may be automated by assembling the micro-columns into an array (e.g. with 96 micro-column arrays).

5        A variety of materials may be used as the source of potential interacting proteins. In one embodiment, a cellular extract or extracellular fluid may be used. The choice of starting material for the extract may be based upon the cell or tissue type or type of fluid that would be expected to contain proteins that interact with the target protein. Micro-organisms or other organisms are grown in a medium that is appropriate for that organism  
10      and can be grown in specific conditions to promote the expression of proteins that may interact with the target protein. Exemplary starting material that may be used to make a suitable extract are: 1) one or more types of tissue derived from an animal, plant, or other multi-cellular organism, 2) cells grown in tissue culture that were derived from an animal or human, plant or other source, 3) micro-organisms grown in suspension or non-suspension  
15      cultures, 4) virus-infected cells, 5) purified organelles (including, but not restricted to nuclei, mitochondria, membranes, Golgi, endoplasmic reticulum, lysosomes, or peroxisomes) prepared by differential centrifugation or another procedure from animal, plant or other kinds of eukaryotic cells, 6) serum or other bodily fluids including, but not limited to, blood, urine, semen, synovial fluid, cerebrospinal fluid, amniotic fluid,  
20      lymphatic fluid or interstitial fluid. In other embodiments, a total cell extract may not be the optimal source of interacting proteins. For example, if the ligand is known to act in the nucleus, a nuclear extract can provide a 10-fold enrichment of proteins that are likely to interact with the ligand. In addition, proteins that are present in the extract in low concentrations may be enriched using another chromatographic method to fractionate the  
25      extract before screening various pools for an interacting protein.

Extracts are prepared by methods known to those of skill in the art. The extracts may be prepared at a low temperature (e.g., 4°C) in order to retard denaturation or degradation of proteins in the extract. The pH of the extract may be adjusted to be appropriate for the body fluid or tissue, cellular, or organellar source that is used for the  
30      procedure (e.g. pH 7-8 for cytosolic extracts from mammals, but low pH for lysosomal extracts). The concentration of chaotropic or non-chaotropic salts in the extracting solution may be adjusted so as to extract the appropriate sets of proteins for the procedure. Glycerol may be added to the extract, as it aids in maintaining the stability of many proteins and also

reduces background non-specific binding. Both the lysis buffer and column buffer may contain protease inhibitors to minimize proteolytic degradation of proteins in the extract and to protect the polypeptide. Appropriate co-factors that could potentially interact with the interacting proteins may be added to the extracting solution. One or more nucleases or 5 another reagent may be added to the extract, if appropriate, to prevent protein-protein interactions that are mediated by nucleic acids. Appropriate detergents or other agents may be added to the solution, if desired, to extract membrane proteins from the cells or tissue. A reducing agent (e.g. dithiothreitol or 2-mercaptoethanol or glutathione or other agent) may be added. Trace metals or a chelating agent may be added, if desired, to the extracting 10 solution.

Usually, the extract is centrifuged in a centrifuge or ultracentrifuge or filtered to provide a clarified supernatant solution. This supernatant solution may be dialyzed using dialysis tubing, or another kind of device that is standard in the art, against a solution that is similar to, but may not be identical with, the solution that was used to make the extract. 15 The extract is clarified by centrifugation or filtration again immediately prior to its use in affinity chromatography.

In some cases, the crude lysate will contain small molecules that can interfere with the affinity chromatography. This can be remedied by precipitating proteins with ammonium sulfate, centrifugation of the precipitate, and re-suspending the proteins in the 20 affinity column buffer followed by dialysis. An additional centrifugation of the sample may be needed to remove any particulate matter prior to application to the affinity columns.

The amount of cell extract applied to the column may be important for any embodiment. If too little extract is applied to the column and the interacting protein is present at low concentration, the level of interacting protein retained by the column may be 25 difficult to detect. Conversely, if too much extract is applied to the column, protein may precipitate on the column or competition by abundant interacting proteins for the limited amount of protein ligand may result in a difficulty in detecting minor species.

The columns functionalized with a polypeptide of the invention are loaded with protein extract from an appropriate source that has been dialyzed against a buffer that is 30 consistent with the nature of the expected interaction. The pH, salt concentrations and the presence or absence of reducing and chelating agents, trace metals, detergents, and co-factors may be adjusted according to the nature of the expected interaction. Most commonly, the pH and the ionic strength are chosen so as to be close to physiological for

the source of the extract. The extract is most commonly loaded under gravity onto the columns at a flow rate of about 4-6 column volumes per hour, but this flow rate can be adjusted for particular circumstances in an automated procedure.

The volume of the extract that is loaded on the columns can be varied but is most

5 commonly equivalent to about 5 to 10 column volumes. When large volumes of extract are loaded on the columns, there is often an improvement in the signal-to-noise ratio because more protein from the extract is available to bind to the protein ligand, whereas the background binding of proteins from the extract to the solid support saturates with low amounts of extract.

10 A control column may be included that contains the highest concentration of protein ligand, but buffer rather than extract is loaded onto this column. The elutions (eluates) from this column will contain polypeptide that failed to be attached to the column in a covalent manner, but no proteins that are derived from the extract.

15 The columns may be washed with a buffer appropriate to the nature of the interaction being analyzed, usually, but not necessarily, the same as the loading buffer. An elution buffer with an appropriate pH, glycerol, and the presence or absence of reducing agent, chelating agent, cofactors, and detergents are all important considerations. The columns may be washed with anywhere from about 5 to 20 column volumes of each wash buffer to eliminate unbound proteins from the natural extract. The flow rate of the wash is

20 usually adjusted to about 4 to 6 column volumes per hour by using gravity or an automated procedure, but other flow rates are possible in specific circumstances.

In order to elute the proteins that have been retained by the column, the interactions between the extract proteins and the column ligand should be disrupted. This is performed by eluting the column with a solution of salt or detergent. Retention of activity by the

25 eluted proteins may require the presence of glycerol and a buffer of appropriate pH, as well as proper choices of ionic strength and the presence or absence of appropriate reducing agent, chelating agent, trace metals, cofactors, detergents, chaotropic agents, and other reagents. If physical identification of the bound proteins is the objective, the elution may be performed sequentially, first with buffer of high ionic strength and then with buffer

30 containing a protein denaturant, most commonly, but not restricted to sodium dodecyl sulfate (SDS), urea, or guanidine hydrochloride. In certain instances, the column is eluted with a protein denaturant, particularly SDS, for example as a 1% SDS solution. Using only the SDS wash, and omitting the salt wash, may result in SDS-gels that have higher

resolution (sharper bands with less smearing). Also, using only the SDS wash results in half as many samples to analyze. The volume of the eluting solution may be varied but is normally about 2 to 4 column volumes. For 20 ml columns, the flow rate of the eluting procedures are most commonly about 4 to 6 column volumes per hour, under gravity, but  
5 can be varied in an automated procedure.

The proteins from the extract that were bound to and are eluted from the affinity columns may be most easily resolved for identification by an electrophoresis procedure, but this procedure may be modified, replaced by another suitable method, or omitted. Any of the denaturing or non-denaturing electrophoresis procedures that are standard in the art may  
10 be used for this purpose, including SDS-PAGE, gradient gels, capillary electrophoresis, and two-dimensional gels with isoelectric focusing in the first dimension and SDS-PAGE in the second. Typically, the individual components in the column eluent are separated by polyacrylamide gel electrophoresis.

After electrophoresis, protein bands or spots may be visualized using any number of  
15 methods known to those of skill in the art, including staining techniques such as Coomassie blue or silver staining, or some other agent that is standard in the art. Alternatively, autoradiography can be used for visualizing proteins isolated from organisms cultured on media containing a radioactive label, for example  $^{35}\text{SO}_4^{2-}$  or  $^{35}\text{S}$ -methionine, that is incorporated into the proteins. The use of radioactively labeled extract allows a distinction  
20 to be made between extract proteins that were retained by the column and proteolytic fragments of the ligand that may be released from the column.

Protein bands that are derived from the extract (i.e. it did not elute from the control column that was not loaded with protein from the extract) and bound to an experimental column that contained polypeptide covalently attached to the solid support, and did not bind  
25 to a control column that did not contain any polypeptide, may be excised from the stained electrophoretic gel and further characterized.

To identify the protein interactor by mass spectrometry, it may be desirable to reduce the disulfide bonds of the protein followed by alkylation of the free thiols prior to digestion of the protein with protease. The reduction may be performed by treatment of the  
30 gel slice with a reducing agent, for example with dithiothreitol, whereupon, the protein is alkylated by treating the gel slice with a suitable alkylating agent, for example iodoacetamide.

Prior to analysis by mass spectrometry, the protein may be chemically or enzymatically digested. The protein sample in the gel slice may be subjected to *in-gel* digestion. Shevchenko A. et al., Mass Spectrometric Sequencing of Proteins from Silver Stained Polyacrylamide Gels. *Analytical Chemistry* 1996, 58, 850-858. One method of 5 digestion is by treatment with the enzyme trypsin. The resulting peptides are extracted from the gel slice into a buffer.

The peptide fragments may be purified, for example by use of chromatography. A solid support that differentially binds the peptides and not the other compounds derived from the gel slice, the protease reaction or the peptide extract may be used. The peptides 10 may be eluted from the solid support into a small volume of a solution that is compatible with mass spectrometry (e.g. 50% acetonitrile/0.1% trifluoroacetic acid).

The preparation of a protein sample from a gel slice that is suitable for mass spectrometry may also be done by an automated procedure.

Peptide samples derived from gel slices may be analyzed by any one of a variety of 15 techniques in mass spectrometry as further described above. This technique may be used to assign function to an unknown protein based upon the known function of the interacting protein in the same or a homologous/orthologous organism.

Eluates from the affinity chromatography columns may also be analyzed directly without resolution by electrophoretic methods, by proteolytic digestion with a protease in 20 solution, followed by applying the proteolytic digestion products to a reverse phase column and eluting the peptides from the column.

In yet another embodiment, proteins that interact with a polypeptide of the invention may be identified using an interaction trap assay (see also, U.S. Patent NO: 5,283,317; Zervos et al. (1993) *Cell* 72:223-232; Madura et al. (1993) *J Biol Chem* 268:12046-12054; 25 Bartel et al. (1993) *Biotechniques* 14:920-924; and Iwabuchi et al. (1993) *Oncogene* 8:1693-1696).

In another embodiment, a method of the present invention makes use of chimeric genes which express hybrid proteins. To illustrate, a first hybrid gene comprises the coding sequence for a DNA-binding domain of a transcriptional activator fused in frame to the 30 coding sequence for a "bait" protein, e.g., a polypeptide of the invention of sufficient length to bind to a potential interacting protein. The second hybrid protein encodes a transcriptional activation domain fused in frame to a gene encoding a "fish" protein, e.g., a potential interacting protein of sufficient length to interact with a polypeptide of the

invention portion of the bait fusion protein. If the bait and fish proteins are able to interact, e.g., form a protein-protein interaction, they bring into close proximity the two domains of the transcriptional activator. This proximity causes transcription of a reporter gene which is operably linked to a transcriptional regulatory site responsive to the transcriptional activator, and expression of the reporter gene can be detected and used to score for the interaction of the bait and fish proteins.

In accordance with the present invention, the method includes providing a host cell, typically a yeast cell, e.g., *Kluyveromyces lactis*, *Schizosaccharomyces pombe*, *Ustilago maydis*, *Saccharomyces cerevisiae*, *Neurospora crassa*, *Aspergillus niger*, *Aspergillus nidulans*, 10 *Pichia pastoris*, *Candida tropicalis*, and *Hansenula polymorpha*, though most preferably *S cerevisiae* or *S. pombe*. The host cell contains a reporter gene having a binding site for the DNA-binding domain of a transcriptional activator used in the bait protein, such that the reporter gene expresses a detectable gene product when the gene is transcriptionally activated. The first chimeric gene may be present in a chromosome of the host cell, or as 15 part of an expression vector.

The host cell also contains a first chimeric gene which is capable of being expressed in the host cell. The gene encodes a chimeric protein, which comprises (a) a DNA-binding domain that recognizes the responsive element on the reporter gene in the host cell, and (b) a bait protein (e.g., a polypeptide of the invention).

20 A second chimeric gene is also provided which is capable of being expressed in the host cell, and encodes the "fish" fusion protein. In one embodiment, both the first and the second chimeric genes are introduced into the host cell in the form of plasmids. Preferably, however, the first chimeric gene is present in a chromosome of the host cell and the second chimeric gene is introduced into the host cell as part of a plasmid.

25 The DNA-binding domain of the first hybrid protein and the transcriptional activation domain of the second hybrid protein may be derived from transcriptional activators having separable DNA-binding and transcriptional activation domains. For instance, these separate DNA-binding and transcriptional activation domains are known to be found in the yeast GAL4 protein, and are known to be found in the yeast GCN4 and 30 ADR1 proteins. Many other proteins involved in transcription also have separable binding and transcriptional activation domains which make them useful for the present invention, and include, for example, the LexA and VP16 proteins. It will be understood that other (substantially) transcriptionally-inert DNA-binding domains may be used in the subject

constructs; such as domains of ACE1, λcI, lac repressor, jun or fos. In another embodiment, the DNA-binding domain and the transcriptional activation domain may be from different proteins. The use of a LexA DNA binding domain provides certain advantages. For example, in yeast, the LexA moiety contains no activation function and

5 has no known affect on transcription of yeast genes. In addition, use of LexA allows control over the sensitivity of the assay to the level of interaction (see, for example, the Brent *et al.* PCT publication WO94/10300).

In certain embodiments, any enzymatic activity associated with the bait or fish proteins is inactivated, e.g., dominant negative or other mutants of a protein-protein

10 interaction component can be used.

Continuing with the illustrative example, a polypeptide of the invention-mediated interaction, if any, between the bait and fish fusion proteins in the host cell, causes the activation domain to activate transcription of the reporter gene. The method is carried out by introducing the first chimeric gene and the second chimeric gene into the host cell, and

15 subjecting that cell to conditions under which the bait and fish fusion proteins are expressed in sufficient quantity for the reporter gene to be activated. The formation of a protein complex containing a polypeptide of the invention results in a detectable signal produced by the expression of the reporter gene.

In still further embodiments, the protein-protein interaction of interest is generated

20 in whole cells, taking advantage of cell culture techniques to support the subject assay. For example, the protein-protein interaction of interest can be constituted in a prokaryotic or eukaryotic cell culture system. Advantages to generating the protein complex in an intact cell includes the ability to screen for inhibitors of the level or activity of the complex which are functional in an environment more closely approximating that which therapeutic use of

25 the inhibitor would require, including the ability of the agent to gain entry into the cell. Furthermore, certain of the *in vivo* embodiments of the assay are amenable to high throughput analysis of candidate agents.

The components of the protein complex comprising a polypeptide of the invention can be endogenous to the cell selected to support the assay. Alternatively, some or all of

30 the components can be derived from exogenous sources. For instance, fusion proteins can be introduced into the cell by recombinant techniques (such as through the use of an expression vector), as well as by microinjecting the fusion protein itself or mRNA encoding the fusion protein. Moreover, in the whole cell embodiments of the subject assay, the

reporter gene construct can provide, upon expression, a selectable marker. Such embodiments of the subject assay are particularly amenable to high through-put analysis in that proliferation of the cell can provide a simple measure of the protein-protein interaction.

The amount of transcription from the reporter gene may be measured using any 5 method known to those of skill in the art to be suitable. For example, specific mRNA expression may be detected using Northern blots or specific protein product may be identified by a characteristic stain, western blots or an intrinsic activity. In certain embodiments, the product of the reporter gene is detected by an intrinsic activity associated with that product. For instance, the reporter gene may encode a gene product that, by 10 enzymatic activity, gives rise to a detection signal based on color, fluorescence, or luminescence.

The interaction trap assay of the invention may also be used to identify test agents capable of modulating formation of a complex comprising a polypeptide of the invention. In general, the amount of expression from the reporter gene in the presence of the test 15 compound is compared to the amount of expression in the same cell in the absence of the test compound. Alternatively, the amount of expression from the reporter gene in the presence of the test compound may be compared with the amount of transcription in a substantially identical cell that lacks a component of the protein-protein interaction involving a polypeptide of the invention.

20

#### *7. Antibodies*

Another aspect of the invention pertains to antibodies specifically reactive with a polypeptide of the invention. For example, by using peptides based on a polypeptide of the invention, e.g., having an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4 or an 25 immunogenic fragment thereof, antisera or monoclonal antibodies may be made using standard methods. An exemplary immunogenic fragment may contain eight, ten or more consecutive amino acid residues of SEQ ID NO: 2 or SEQ ID NO: 4. Certain fragments that are predicted to be immunogenic for the subject amino acid sequences (predicted) are set forth in Table 2 contained in FIGURE 7

30

The term "antibody" as used herein is intended to include fragments thereof which are also specifically reactive with a polypeptide of the invention. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as is suitable for whole antibodies. For example,  $F(ab')_2$  fragments can be

generated by treating antibody with pepsin. The resulting F(ab')<sub>2</sub> fragment can be treated to reduce disulfide bridges to produce Fab' fragments. The antibody of the present invention is further intended to include bispecific and chimeric molecules, as well as single chain (scFv) antibodies. Also within the scope of the invention are trimeric antibodies, 5 humanized antibodies, human antibodies, and single chain antibodies. All of these modified forms of antibodies as well as fragments of antibodies are intended to be included in the term "antibody".

In one aspect, the present invention contemplates a purified antibody that binds specifically to a polypeptide of the invention and which does not substantially cross-react 10 with a protein which is less than about 80%, or less than about 90%, identical to SEQ ID NO: 2 or SEQ ID NO: 4. In another aspect, the present invention contemplates an array comprising a substrate having a plurality of address, wherein at least one of the addresses has disposed thereon a purified antibody that binds specifically to a polypeptide of the invention.

15       Antibodies may be elicited by methods known in the art. For example, a mammal such as a mouse, a hamster or rabbit may be immunized with an immunogenic form of a polypeptide of the invention (e.g., an antigenic fragment which is capable of eliciting an antibody response). Alternatively, immunization may occur by using a nucleic acid of the acid, which presumably *in vivo* expresses the polypeptide of the invention giving rise to the 20 immunogenic response observed. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. For instance, a peptidyl portion of a polypeptide of the invention may be administered in the presence of adjuvant. The progress of immunization may be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays may be used 25 with the immunogen as antigen to assess the levels of antibodies.

Following immunization, antisera reactive with a polypeptide of the invention may be obtained and, if desired, polyclonal antibodies isolated from the serum. To produce monoclonal antibodies, antibody producing cells (lymphocytes) may be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing 30 cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique (originally developed by Kohler and Milstein, (1975) *Nature*, 256: 495-497), as the human B cell hybridoma technique (Kozbar et al., (1983) *Immunology Today*, 4: 72), and the EBV-hybridoma technique to

produce human monoclonal antibodies (Cole et al., (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. pp. 77-96). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the polypeptides of the invention and the monoclonal antibodies isolated.

5       Antibodies directed against the polypeptides of the invention can be used to selectively block the action of the polypeptides of the invention. Antibodies against a polypeptide of the invention may be employed to treat infections, particularly bacterial infections and diseases. For example, the present invention contemplates a method for treating a subject suffering from a *P. aeruginosa* related disease or disorder, comprising  
10 administering to an animal having the condition a therapeutically effective amount of a purified antibody that binds specifically to a polypeptide of the invention. In another example, the present invention contemplates a method for inhibiting SEQ ID NO: 2 or SEQ ID NO: 4 dependent growth or infectivity of *P. aeruginosa*, comprising contacting *P. aeruginosa* with a purified antibody that binds specifically to a polypeptide of the invention.  
15

In one embodiment, antibodies reactive with a polypeptide of the invention are used in the immunological screening of cDNA libraries constructed in expression vectors, such as  $\lambda$ gt11,  $\lambda$ gt18-23,  $\lambda$ ZAP, and  $\lambda$ ORF8. Messenger libraries of this type, having coding sequences inserted in the correct reading frame and orientation, can produce fusion proteins. For instance,  $\lambda$ gt11 will produce fusion proteins whose amino termini consist of  $\beta$ -galactosidase amino acid sequences and whose carboxy termini consist of a foreign polypeptide. Antigenic epitopes of a polypeptide of the invention can then be detected with antibodies, as, for example, reacting nitrocellulose filters lifted from phage infected bacterial plates with an antibody specific for a polypeptide of the invention. Phage scored by this assay can then be isolated from the infected plate. Thus, homologs of a polypeptide of the invention can be detected and cloned from other sources.

Antibodies may be employed to isolate or to identify clones expressing the polypeptides to purify the polypeptides by affinity chromatography.

In other embodiments, the polypeptides of the invention may be modified so as to increase their immunogenicity. For example, a polypeptide, such as an antigenically or immunologically equivalent derivative, may be associated, for example by conjugation, with an immunogenic carrier protein for example bovine serum albumin (BSA) or keyhole limpet haemocyanin (KLH). Alternatively a multiple antigenic peptide comprising multiple

copies of the protein or polypeptide, or an antigenically or immunologically equivalent polypeptide thereof may be sufficiently antigenic to improve immunogenicity so as to obviate the use of a carrier.

In other embodiments, the antibodies of the invention, or variants thereof, are modified to make them less immunogenic when administered to a subject. For example, if the subject is human, the antibody may be "humanized"; where the complementarity determining region(s) of the hybridoma-derived antibody has been transplanted into a human monoclonal antibody, for example as described in Jones, P. et al. (1986), Nature 321, 522-525 or Tempest et al. (1991) Biotechnology 9, 266-273. Also, transgenic mice, or other mammals, may be used to express humanized antibodies. Such humanization may be partial or complete.

The use of a nucleic acid of the invention in genetic immunization may employ a suitable delivery method such as direct injection of plasmid DNA into muscles (Wolff et al., Hum Mol Genet 1992, 1:363, Manthorpe et al., Hum. Gene Ther. 1963:4, 419), delivery of DNA complexed with specific protein carriers (Wu et al., J Biol Chem. 1989: 264,16985), coprecipitation of DNA with calcium phosphate (Benvenisty & Reshef, PNAS USA, 1986:83,9551), encapsulation of DNA in various forms of liposomes (Kaneda et al., Science 1989:243,375), particle bombardment (Tang et al., Nature 1992, 356:152, Eisenbraun et al., DNA Cell Biol 1993, 12:791) and *in vivo* infection using cloned retroviral vectors (Seeger et al., PNAS USA 1984:81,5849).

#### *8. Diagnostic Assays*

The invention further provides a method for detecting the presence of *P. aeruginosa* in a biological sample. Detection of *P. aeruginosa* in a subject, particularly a mammal, and especially a human, will provide a diagnostic method for diagnosis of a *P. aeruginosa* related disease or disorder. In general, the method involves contacting the biological sample with a compound or an agent capable of detecting a polypeptide of the invention or a nucleic acid of the invention. The term "biological sample" when used in reference to a diagnostic assay is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject.

The detection method of the invention may be used to detect the presence of *P. aeruginosa* in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of a nucleic acid of the invention include Northern hybridizations

and *in situ* hybridizations. *In vitro* techniques for detection of polypeptides of the invention include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, immunofluorescence, radioimmunoassays and competitive binding assays. Alternatively, polypeptides of the invention can be detected *in vivo* in a subject by

5 introducing into the subject a labeled antibody specific for a polypeptide of the invention. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. It may be possible to use all of the diagnostic methods disclosed herein for pathogens in addition to *P. aeruginosa*.

10 Nucleic acids for diagnosis may be obtained from an infected individual's cells and tissues, such as bone, blood, muscle, cartilage, and skin. Nucleic acids, e.g., DNA and RNA, may be used directly for detection or may be amplified, e.g., enzymatically by using PCR or other amplification technique, prior to analysis. Using amplification, characterization of the species and strain of prokaryote present in an individual, may be made by an analysis of the genotype of the prokaryote gene. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the genotype of a reference sequence. Point mutations can be identified by hybridizing a nucleic acid, e.g., amplified DNA, to a nucleic acid of the invention, which nucleic acid may be labeled. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase 15 digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in the electrophoretic mobility of the DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g. Myers et al., Science, 230: 1242 (1985). Sequence changes at specific locations also may be revealed by nuclelease protection assays, such as RNase and S1 protection or a chemical cleavage 20 method. See, e.g., Cotton et al., Proc. Natl. Acad. Sci., USA, 85: 4397-4401 (1985).

Agents for detecting a nucleic acid of the invention, e.g., comprising the sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 3, include labeled or labelable nucleic acid probes capable of hybridizing to a nucleic acid of the invention. The nucleic acid probe can comprise, for example, the full length sequence of a nucleic acid of the invention, or an equivalent thereof, or a portion thereof, such as an oligonucleotide of at least 15, 30, 50, 30 100, 250 or 500 nucleotides in length and sufficient to specifically hybridize under stringent conditions to SEQ ID NO: 1 or SEQ ID NO: 3, or the complement thereof. Agents for detecting a polypeptide of the invention, e.g., comprising an amino acid sequence of SEQ

ID NO: 2 or SEQ ID NO: 4, include labeled or labelable antibodies capable of binding to a polypeptide of the invention. Antibodies may be polyclonal, or alternatively, monoclonal. An intact antibody, or a fragment thereof (e.g., Fab or F(ab')<sub>2</sub>) can be used. Labeling the probe or antibody also encompasses direct labeling of the probe or antibody by coupling (e.g., physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently labeled streptavidin.

In certain embodiments, detection of a nucleic acid of the invention in a biological sample involves the use of a probe/primer in a polymerase chain reaction (PCR) (see, e.g. U.S. Pat. Nos. 4,683,195 and 4,683,202), such as anchor PCR or RACE PCR, or, alternatively, in a ligation chain reaction (LCR) (see, e.g., Landegran et al. (1988) Science 241:1077-1080; and Nakazawa et al. (1994) PNAS 91:360-364), the latter of which can be particularly useful for distinguishing between orthologs of polynucleotides of the invention (see Abravaya et al. (1995) Nucleic Acids Res. 23:675-682). This method can include the steps of collecting a sample of cells from a patient, isolating nucleic acid (e.g., genomic, mRNA or both) from the cells of the sample, contacting the nucleic acid sample with one or more primers which specifically hybridize to a nucleic acid of the invention under conditions such that hybridization and amplification of the polynucleotide (if present) occurs, and detecting the presence or absence of an amplification product, or detecting the size of the amplification product and comparing the length to a control sample.

In one aspect, the present invention contemplates a method for detecting the presence of *P. aeruginosa* in a sample, the method comprising: (a) providing a sample to be tested for the presence of *P. aeruginosa*; (b) contacting the sample with an antibody reactive against eight consecutive amino acid residues of SEQ ID NO: 2 or SEQ ID NO: 4 under conditions which permit association between the antibody and its ligand; and (c) detecting interaction of the antibody with its ligand, thereby detecting the presence of *P. aeruginosa* in the sample.

In another aspect, the present invention contemplates a method for detecting the presence of *P. aeruginosa* in a sample, the method comprising: (a) providing a sample to be tested for the presence of *P. aeruginosa*; (b) contacting the sample with an antibody that binds specifically to a polypeptide of the invention under conditions which permit

association between the antibody and its ligand; and (c) detecting interaction of the antibody with its ligand, thereby detecting the presence of *P. aeruginosa* in the sample.

In yet another example, the present invention contemplates a method for diagnosing a patient suffering from a *P. aeruginosa* related disease or disorder, comprising:

5 (a) obtaining a biological sample from a patient; (b) detecting the presence or absence of a polypeptide of the invention, or a nucleic acid encoding a polypeptide of the invention, in the sample; and (c) diagnosing a patient suffering from a *P. aeruginosa* related disease or disorder based on the presence of a polypeptide of the invention, or a nucleic acid encoding a polypeptide of the invention, in the patient sample.

10 The diagnostic assays of the invention may also be used to monitor the effectiveness of an anti-*P. aeruginosa* treatment in an individual suffering from an *P. aeruginosa* related disease or disorder. For example, the presence and/or amount of a nucleic acid of the invention or a polypeptide of the invention can be detected in an individual suffering from an *P. aeruginosa* related disease or disorder before and after treatment with anti-*P.*

15 *aeruginosa* therapeutic agent. Any change in the level of a polynucleotide or polypeptide of the invention after treatment of the individual with the therapeutic agent can provide information about the effectiveness of the treatment course. In particular, no change, or a decrease, in the level of a polynucleotide or polypeptide of the invention present in the biological sample will indicate that the therapeutic is successfully combating the *P. aeruginosa* related disease or disorder.

20 The invention also encompasses kits for detecting the presence of *P. aeruginosa* in a biological sample. For example, the kit can comprise a labeled or labelable compound or agent capable of detecting a polynucleotide or polypeptide of the invention in a biological sample; means for determining the amount of *P. aeruginosa* in the sample; and means for 25 comparing the amount of *P. aeruginosa* in the sample with a standard. The compound or agent can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect a polynucleotide or polypeptide of the invention.

#### *9. Drug Discovery*

30 Modulators to polypeptides of the invention and other structurally related molecules, and complexes containing the same, may be identified and developed as set forth below and otherwise using techniques and methods known to those of skill in the art. The modulators of the invention may be employed, for instance, to inhibit and treat *P.*

*aeruginosa* associated diseases or conditions, such as osteomyelitis, otitis externa, conjunctivitis, keratitis, endophthalmitis, alveolar necrosis, vascular invasion, bacteremia, and burn infection.

5 A variety of methods for inhibiting the growth or infectivity of *P. aeruginosa* are contemplated by the present invention. For example, exemplary methods involve contacting *P. aeruginosa* with a polypeptide of the invention that modulates the same or another polypeptide from such pathogen, a nucleic acid encoding such polypeptide of the invention, or a compound thought or shown to be effective against such pathogen.

10 For example, in one aspect, the present invention contemplates a method for treating a patient suffering from an infection of *P. aeruginosa*, comprising administering to the patient an amount of a SEQ ID NO: 2 or SEQ ID NO: 4 inhibitor effective to inhibit the expression and/or activity of a polypeptide of the invention. In certain instances, the animal is a human or a livestock animal such as a cow, pig, goat or sheep. The present invention further contemplates a method for treating a subject suffering from a *P. aeruginosa* related 15 disease or disorder, comprising administering to an animal having the condition a therapeutically effective amount of a molecule identified using one of the methods of the present invention.

The present invention contemplates making any molecule that is shown to modulate the activity of a polypeptide of the invention.

20 In another embodiment, inhibitors, modulators of the subject polypeptides, or biological complexes containing them, may be used in the manufacture of a medicament for any number of uses, including, for example, treating any disease or other treatable condition of a patient (including humans and animals), and particularly a disease caused by *P. aeruginosa*, such as, for example, one of the following: osteomyelitis, otitis externa, conjunctivitis, keratitis, endophthalmitis, alveolar necrosis, vascular invasion, bacteremia, 25 and burn infection.

#### (a) Drug Design

A number of techniques can be used to screen, identify, select and design chemical entities capable of associating with polypeptides of the invention, structurally homologous 30 molecules, and other molecules. Knowledge of the structure for a polypeptide of the invention, determined in accordance with the methods described herein, permits the design and/or identification of molecules and/or other modulators which have a shape complementary to the conformation of a polypeptide of the invention, or more particularly,

a druggable region thereof. It is understood that such techniques and methods may use, in addition to the exact structural coordinates and other information for a polypeptide of the invention, structural equivalents thereof described above (including, for example, those structural coordinates that are derived from the structural coordinates of amino acids 5 contained in a druggable region as described above).

The term "chemical entity," as used herein, refers to chemical compounds, complexes of two or more chemical compounds, and fragments of such compounds or complexes. In certain instances, it is desirable to use chemical entities exhibiting a wide range of structural and functional diversity, such as compounds exhibiting different shapes 10 (e.g., flat aromatic rings(s), puckered aliphatic rings(s), straight and branched chain aliphatics with single, double, or triple bonds) and diverse functional groups (e.g., carboxylic acids, esters, ethers, amines, aldehydes, ketones, and various heterocyclic rings).

In one aspect, the method of drug design generally includes computationally evaluating the potential of a selected chemical entity to associate with any of the molecules 15 or complexes of the present invention (or portions thereof). For example, this method may include the steps of (a) employing computational means to perform a fitting operation between the selected chemical entity and a druggable region of the molecule or complex; and (b) analyzing the results of said fitting operation to quantify the association between the chemical entity and the druggable region.

A chemical entity may be examined either through visual inspection or through the use of computer modeling using a docking program such as GRAM, DOCK, or AUTODOCK (Dunbrack et al., *Folding & Design*, 2:27-42 (1997)). This procedure can include computer fitting of chemical entities to a target to ascertain how well the shape and the chemical structure of each chemical entity will complement or interfere with the 20 structure of the subject polypeptide (Bugg et al., *Scientific American*, Dec.: 92-98 (1993); West et al., *TIPS*, 16:67-74 (1995)). Computer programs may also be employed to estimate the attraction, repulsion, and steric hindrance of the chemical entity to a druggable region, for example. Generally, the tighter the fit (e.g., the lower the steric hindrance, and/or the greater the attractive force) the more potent the chemical entity will be because these 25 properties are consistent with a tighter binding constant. Furthermore, the more specificity in the design of a chemical entity the more likely that the chemical entity will not interfere with related proteins, which may minimize potential side-effects due to unwanted interactions.

A variety of computational methods for molecular design, in which the steric and electronic properties of druggable regions are used to guide the design of chemical entities, are known: Cohen et al. (1990) *J. Med. Chem.* 33: 883-894; Kuntz et al. (1982) *J. Mol. Biol.* 161: 269-288; DesJarlais (1988) *J. Med. Chem.* 31: 722-729; Bartlett et al. (1989) *Spec. Publ., Roy. Soc. Chem.* 78: 182-196; Goodford et al. (1985) *J. Med. Chem.* 28: 849-857; and DesJarlais et al. *J. Med. Chem.* 29: 2149-2153. Directed methods generally fall into two categories: (1) design by analogy in which 3-D structures of known chemical entities (such as from a crystallographic database) are docked to the druggable region and scored for goodness-of-fit; and (2) *de novo* design, in which the chemical entity is constructed piece-wise in the druggable region. The chemical entity may be screened as part of a library or a database of molecules. Databases which may be used include ACD (Molecular Designs Limited), NCI (National Cancer Institute), CCDC (Cambridge Crystallographic Data Center), CAST (Chemical Abstract Service), Derwent (Derwent Information Limited), Maybridge (Maybridge Chemical Company Ltd), Aldrich (Aldrich Chemical Company), DOCK (University of California in San Francisco), and the Directory of Natural Products (Chapman & Hall). Computer programs such as CONCORD (Tripos Associates) or DB-Converter (Molecular Simulations Limited) can be used to convert a data set represented in two dimensions to one represented in three dimensions.

Chemical entities may be tested for their capacity to fit spatially with a druggable region or other portion of a target protein. As used herein, the term "fits spatially" means that the three-dimensional structure of the chemical entity is accommodated geometrically by a druggable region. A favorable geometric fit occurs when the surface area of the chemical entity is in close proximity with the surface area of the druggable region without forming unfavorable interactions. A favorable complementary interaction occurs where the chemical entity interacts by hydrophobic, aromatic, ionic, dipolar, or hydrogen donating and accepting forces. Unfavorable interactions may be steric hindrance between atoms in the chemical entity and atoms in the druggable region.

If a model of the present invention is a computer model, the chemical entities may be positioned in a druggable region through computational docking. If, on the other hand, the model of the present invention is a structural model, the chemical entities may be positioned in the druggable region by, for example, manual docking. As used herein the term "docking" refers to a process of placing a chemical entity in close proximity with a

druggable region, or a process of finding low energy conformations of a chemical entity/druggable region complex.

In an illustrative embodiment, the design of potential modulator begins from the general perspective of shape complimentary for the druggable region of a polypeptide of 5 the invention, and a search algorithm is employed which is capable of scanning a database of small molecules of known three-dimensional structure for chemical entities which fit geometrically with the target druggable region. Most algorithms of this type provide a method for finding a wide assortment of chemical entities that are complementary to the shape of a druggable region of the subject polypeptide. Each of a set of chemical entities 10 from a particular data-base, such as the Cambridge Crystallographic Data Bank (CCDB) (Allen et al. (1973) *J. Chem. Doc.* 13: 119), is individually docked to the druggable region of a polypeptide of the invention in a number of geometrically permissible orientations with use of a docking algorithm. In certain embodiments, a set of computer algorithms called DOCK, can be used to characterize the shape of invaginations and grooves that form the 15 active sites and recognition surfaces of the druggable region (Kuntz et al. (1982) *J. Mol. Biol.* 161: 269-288). The program can also search a database of small molecules for templates whose shapes are complementary to particular binding sites of a polypeptide of the invention (DesJarlais et al. (1988) *J Med Chem* 31: 722-729).

The orientations are evaluated for goodness-of-fit and the best are kept for further 20 examination using molecular mechanics programs, such as AMBER or CHARMM. Such algorithms have previously proven successful in finding a variety of chemical entities that are complementary in shape to a druggable region.

Goodford (1985, *J Med Chem* 28:849-857) and Boobbyer et al. (1989, *J Med Chem* 32:1083-1094) have produced a computer program (GRID) which seeks to determine regions 25 of high affinity for different chemical groups (termed probes) of the druggable region. GRID hence provides a tool for suggesting modifications to known chemical entities that might enhance binding. It may be anticipated that some of the sites discerned by GRID as regions of high affinity correspond to "pharmacophoric patterns" determined inferentially from a series of known ligands. As used herein, a "pharmacophoric pattern" is a geometric arrangement of 30 features of chemical entities that is believed to be important for binding. Attempts have been made to use pharmacophoric patterns as a search screen for novel ligands (Jakes et al. (1987) *J Mol Graph* 5:41-48; Brint et al. (1987) *J Mol Graph* 5:49-56; Jakes et al. (1986) *J Mol Graph* 4:12-20).

Yet a further embodiment of the present invention utilizes a computer algorithm such as CLIX which searches such databases as CCDB for chemical entities which can be oriented with the druggable region in a way that is both sterically acceptable and has a high likelihood of achieving favorable chemical interactions between the chemical entity and the surrounding 5 amino acid residues. The method is based on characterizing the region in terms of an ensemble of favorable binding positions for different chemical groups and then searching for orientations of the chemical entities that cause maximum spatial coincidence of individual candidate chemical groups with members of the ensemble. The algorithmic details of CLIX is described in Lawrence et al. (1992) *Proteins* 12:31-41.

10 In this way, the efficiency with which a chemical entity may bind to or interfere with a druggable region may be tested and optimized by computational evaluation. For example, for a favorable association with a druggable region, a chemical entity must preferably demonstrate a relatively small difference in energy between its bound and free states (i.e., a small deformation energy of binding). Thus, certain, more desirable chemical 15 entities will be designed with a deformation energy of binding of not greater than about 10 kcal/mole, and more preferably, not greater than 7 kcal/mole. Chemical entities may interact with a druggable region in more than one conformation that is similar in overall binding energy. In those cases, the deformation energy of binding is taken to be the difference between the energy of the free entity and the average energy of the 20 conformations observed when the chemical entity binds to the target.

In this way, the present invention provides computer-assisted methods for identifying or designing a potential modulator of the activity of a polypeptide of the invention including: supplying a computer modeling application with a set of structure coordinates of a molecule or complex, the molecule or complex including at least a portion 25 of a druggable region from a polypeptide of the invention; supplying the computer modeling application with a set of structure coordinates of a chemical entity; and determining whether the chemical entity is expected to bind to the molecule or complex, wherein binding to the molecule or complex is indicative of potential modulation of the activity of a polypeptide of the invention.

30 In another aspect, the present invention provides a computer-assisted method for identifying or designing a potential modulator to a polypeptide of the invention, supplying a computer modeling application with a set of structure coordinates of a molecule or complex, the molecule or complex including at least a portion of a druggable region of a

polypeptide of the invention; supplying the computer modeling application with a set of structure coordinates for a chemical entity; evaluating the potential binding interactions between the chemical entity and active site of the molecule or molecular complex; structurally modifying the chemical entity to yield a set of structure coordinates for a modified chemical entity, and determining whether the modified chemical entity is expected to bind to the molecule or complex, wherein binding to the molecule or complex is indicative of potential modulation of the polypeptide of the invention.

In one embodiment, a potential modulator can be obtained by screening a peptide library (Scott and Smith, *Science*, 249:386-390 (1990); Cwirla et al., *Proc. Natl. Acad. Sci.*, 87:6378-6382 (1990); Devlin et al., *Science*, 249:404-406 (1990)). A potential modulator selected in this manner could then be systematically modified by computer modeling programs until one or more promising potential drugs are identified. Such analysis has been shown to be effective in the development of HIV protease inhibitors (Lam et al., *Science* 263:380-384 (1994); Wlodawer et al., *Ann. Rev. Biochem.* 62:543-585 (1993); Appelt, *Perspectives in Drug Discovery and Design* 1:23-48 (1993); Erickson, *Perspectives in Drug Discovery and Design* 1:109-128 (1993)). Alternatively a potential modulator may be selected from a library of chemicals such as those that can be licensed from third parties, such as chemical and pharmaceutical companies. A third alternative is to synthesize the potential modulator *de novo*.

For example, in certain embodiments, the present invention provides a method for making a potential modulator for a polypeptide of the invention, the method including synthesizing a chemical entity or a molecule containing the chemical entity to yield a potential modulator of a polypeptide of the invention, the chemical entity having been identified during a computer-assisted process including supplying a computer modeling application with a set of structure coordinates of a molecule or complex, the molecule or complex including at least one druggable region from a polypeptide of the invention; supplying the computer modeling application with a set of structure coordinates of a chemical entity; and determining whether the chemical entity is expected to bind to the molecule or complex at the active site, wherein binding to the molecule or complex is indicative of potential modulation. This method may further include the steps of evaluating the potential binding interactions between the chemical entity and the active site of the molecule or molecular complex and structurally modifying the chemical entity to yield a set

of structure coordinates for a modified chemical entity, which steps may be repeated one or more times.

Once a potential modulator is identified, it can then be tested in any standard assay for the macromolecule depending of course on the macromolecule, including in high throughput assays. Further refinements to the structure of the modulator will generally be necessary and can be made by the successive iterations of any and/or all of the steps provided by the particular screening assay, in particular further structural analysis by e.g., <sup>15</sup>N NMR relaxation rate determinations or x-ray crystallography with the modulator bound to the subject polypeptide. These studies may be performed in conjunction with biochemical assays.

Once identified, a potential modulator may be used as a model structure, and analogs to the compound can be obtained. The analogs are then screened for their ability to bind the subject polypeptide. An analog of the potential modulator might be chosen as a modulator when it binds to the subject polypeptide with a higher binding affinity than the predecessor modulator.

In a related approach, iterative drug design is used to identify modulators of a target protein. Iterative drug design is a method for optimizing associations between a protein and a modulator by determining and evaluating the three dimensional structures of successive sets of protein/modulator complexes. In iterative drug design, crystals of a series of protein/modulator complexes are obtained and then the three-dimensional structures of each complex is solved. Such an approach provides insight into the association between the proteins and modulators of each complex. For example, this approach may be accomplished by selecting modulators with inhibitory activity, obtaining crystals of this new protein/modulator complex, solving the three dimensional structure of the complex, and comparing the associations between the new protein/modulator complex and previously solved protein/modulator complexes. By observing how changes in the modulator affected the protein/modulator associations, these associations may be optimized.

In addition to designing and/or identifying a chemical entity to associate with a druggable region, as described above, the same techniques and methods may be used to design and/or identify chemical entities that either associate, or do not associate, with affinity regions, selectivity regions or undesired regions of protein targets. By such methods, selectivity for one or a few targets, or alternatively for multiple targets, from the same species or from multiple species, can be achieved.

For example, a chemical entity may be designed and/or identified for which the binding energy for one druggable region, e.g., an affinity region or selectivity region, is more favorable than that for another region, e.g., an undesired region, by about 20%, 30%, 50% to about 60% or more. It may be the case that the difference is observed between

5       (a) more than two regions, (b) between different regions (selectivity, affinity or undesirable) from the same target, (c) between regions of different targets, (d) between regions of homologs from different species, or (e) between other combinations. Alternatively, the comparison may be made by reference to the  $K_d$ , usually the apparent  $K_d$ , of said chemical entity with the two or more regions in question.

10      In another aspect, prospective modulators are screened for binding to two nearby druggable regions on a target protein. For example, a modulator that binds a first region of a target polypeptide does not bind a second nearby region. Binding to the second region can be determined by monitoring changes in a different set of amide chemical shifts in either the original screen or a second screen conducted in the presence of a modulator (or  
15     potential modulator) for the first region. From an analysis of the chemical shift changes, the approximate location of a potential modulator for the second region is identified. Optimization of the second modulator for binding to the region is then carried out by screening structurally related compounds (e.g., analogs as described above). When modulators for the first region and the second region are identified, their location and  
20     orientation in the ternary complex can be determined experimentally. On the basis of this structural information, a linked compound, e.g., a consolidated modulator, is synthesized in which the modulator for the first region and the modulator for the second region are linked. In certain embodiments, the two modulators are covalently linked to form a consolidated modulator. This consolidated modulator may be tested to determine if it has a higher  
25     binding affinity for the target than either of the two individual modulators. A consolidated modulator is selected as a modulator when it has a higher binding affinity for the target than either of the two modulators. Larger consolidated modulators can be constructed in an analogous manner, e.g., linking three modulators which bind to three nearby regions on the target to form a multilinked consolidated modulator that has an even higher affinity for the  
30     target than the linked modulator. In this example, it is assumed that is desirable to have the modulator bind to all the druggable regions. However, it may be the case that binding to certain of the druggable regions is not desirable, so that the same techniques may be used to

identify modulators and consolidated modulators that show increased specificity based on binding to at least one but not all druggable regions of a target.

The present invention provides a number of methods that use drug design as described above. For example, in one aspect, the present invention contemplates a method

5 for designing a candidate compound for screening for inhibitors of a polypeptide of the invention, the method comprising: (a) determining the three dimensional structure of a crystallized polypeptide of the invention or a fragment thereof; and (b) designing a candidate inhibitor based on the three dimensional structure of the crystallized polypeptide or fragment.

10 In another aspect, the present invention contemplates a method for identifying a potential inhibitor of a polypeptide of the invention, the method comprising: (a) providing the three-dimensional coordinates of a polypeptide of the invention or a fragment thereof; (b) identifying a druggable region of the polypeptide or fragment; and (c) selecting from a database at least one compound that comprises three dimensional coordinates which indicate that the compound may bind the druggable region; (d) wherein the selected 15 compound is a potential inhibitor of a polypeptide of the invention.

In another aspect, the present invention contemplates a method for identifying a potential modulator of a molecule comprising a druggable region similar to that of SEQ ID NO: 2 or SEQ ID NO: 4, the method comprising: (a) using the atomic coordinates of amino 20 acid residues from SEQ ID NO: 2 or SEQ ID NO: 4, or a fragment thereof,  $\pm$  a root mean square deviation from the backbone atoms of the amino acids of not more than 1.5 Å, to generate a three-dimensional structure of a molecule comprising a druggable region that is a portion of SEQ ID NO: 2 or SEQ ID NO: 4; (b) employing the three dimensional structure to design or select the potential modulator; (c) synthesizing the modulator; and 25 (d) contacting the modulator with the molecule to determine the ability of the modulator to interact with the molecule.

In another aspect, the present invention contemplates an apparatus for determining whether a compound is a potential inhibitor of a polypeptide having SEQ ID NO: 2 or SEQ ID NO: 4, the apparatus comprising: (a) a memory that comprises: (i) the three dimensional 30 coordinates and identities of the atoms of a polypeptide of the invention or a fragment thereof that form a druggable site; and (ii) executable instructions; and (b) a processor that is capable of executing instructions to: (i) receive three-dimensional structural information for a candidate compound; (ii) determine if the three-dimensional structure of the candidate

compound is complementary to the structure of the interior of the druggable site; and (iii) output the results of the determination.

In another aspect, the present invention contemplates a method for designing a potential compound for the prevention or treatment of *P. aeruginosa* related disease or disorder, the method comprising: (a) providing the three dimensional structure of a crystallized polypeptide of the invention, or a fragment thereof; (b) synthesizing a potential compound for the prevention or treatment of *P. aeruginosa* related disease or disorder based on the three dimensional structure of the crystallized polypeptide or fragment; (c) contacting a polypeptide of the present invention or an *P. aeruginosa* with the potential compound; and (d) assaying the activity of a polypeptide of the present invention, wherein a change in the activity of the polypeptide indicates that the compound may be useful for prevention or treatment of a *P. aeruginosa* related disease or disorder.

In another aspect, the present invention contemplates a method for designing a potential compound for the prevention or treatment of *P. aeruginosa* related disease or disorder, the method comprising: (a) providing structural information of a druggable region derived from NMR spectroscopy of a polypeptide of the invention, or a fragment thereof; (b) synthesizing a potential compound for the prevention or treatment of *P. aeruginosa* related disease or disorder based on the structural information; (c) contacting a polypeptide of the present invention or an *P. aeruginosa* with the potential compound; and (d) assaying the activity of a polypeptide of the present invention, wherein a change in the activity of the polypeptide indicates that the compound may be useful for prevention or treatment of a *P. aeruginosa* related disease or disorder.

*(b) In Vitro Assays*

Polypeptides of the invention may be used to assess the activity of small molecules and other modulators in *in vitro* assays. In one embodiment of such an assay, agents are identified which modulate the biological activity of a protein, protein-protein interaction of interest or protein complex, such as an enzymatic activity, binding to other cellular components, cellular compartmentalization, signal transduction, and the like. In certain embodiments, the test agent is a small organic molecule.

Assays may employ kinetic or thermodynamic methodology using a wide variety of techniques including, but not limited to, microcalorimetry, circular dichroism, capillary zone electrophoresis, nuclear magnetic resonance spectroscopy, fluorescence spectroscopy, and combinations thereof.

The invention also provides a method of screening compounds to identify those which modulate the action of polypeptides of the invention, or polynucleotides encoding the same. The method of screening may involve high-throughput techniques. For example, to screen for modulators, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising a polypeptide of the invention and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a modulator of a polypeptide of the invention. The ability of the candidate molecule to modulate a polypeptide of the invention is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Detection of the rate or level of production of product from substrate may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric labeled substrate converted into product, a reporter gene that is responsive to changes in a nucleic acid of the invention or polypeptide activity, and binding assays known in the art.

Another example of an assay for a modulator of a polypeptide of the invention is a competitive assay that combines a polypeptide of the invention and a potential modulator with molecules that bind to a polypeptide of the invention, recombinant molecules that bind to a polypeptide of the invention, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. Polypeptides of the invention can be labeled, such as by radioactivity or a colorimetric compound, such that the number of molecules of a polypeptide of the invention bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential modulator.

A number of methods for identifying a molecule which modulates the activity of a polypeptide are known in the art. For example, in one such method, a subject polypeptide is contacted with a test compound, and the activity of the subject polypeptide in the presence of the test compound is determined, wherein a change in the activity of the subject polypeptide is indicative that the test compound modulates the activity of the subject polypeptide. In certain instances, the test compound agonizes the activity of the subject polypeptide, and in other instances, the test compound antagonizes the activity of the subject polypeptide.

In another example, a compound which modulates SEQ ID NO: 2 or SEQ ID NO: 4 dependent growth or infectivity of *P. aeruginosa* may be identified by (a) contacting a polypeptide of the invention with a test compound; and (b) determining the activity of the polypeptide in the presence of the test compound, wherein a change in the activity of the polypeptide is indicative that the test compound may modulate the growth or infectivity of *P. aeruginosa*.

*(c) In Vivo Assays*

Animal models of bacterial infection and/or disease may be used as an *in vivo* assay for evaluating the effectiveness of a potential drug target in treating or preventing diseases or disorders. A number of suitable animal models are described briefly below, however, these models are only examples and modifications, or completely different animal models, may be used in accord with the methods of the invention.

*(i) Mouse Soft Tissue Model*

The mouse soft tissue infection model is a sensitive and effective method for measurement of bacterial proliferation. In these models (Vogelman et al., 1988, J. Infect. Dis. 157: 287-298) anesthetized mice are infected with the bacteria in the muscle of the hind thigh. The mice can be either chemically immune compromised (e.g., cytoxin treated at 125 mg/kg on days -4, -2, and 0) or immunocompetent. The dose of microbe necessary to cause an infection is variable and depends on the individual microbe, but commonly is on the order of  $10^5$  -  $10^6$  colony forming units per injection for bacteria. A variety of mouse strains are useful in this model although Swiss Webster and DBA2 lines are most commonly used. Once infected the animals are conscious and show no overt ill effects of the infections for approximately 12 hours. After that time virulent strains cause swelling of the thigh muscle, and the animals can become bacteremic within approximately 24 hours.

This model most effectively measures proliferation of the microbe, and this proliferation is measured by sacrifice of the infected animal and counting colonies from homogenized thighs.

*(ii) Diffusion Chamber Model*

A second model useful for assessing the virulence of microbes is the diffusion chamber model (Malouin et al., 1990, Infect. Immun. 58: 1247-1253; Doy et al., 1980, J. Infect. Dis. 2: 39-51; Kelly et al., 1989, Infect. Immun. 57: 344-350). In this model rodents have a diffusion chamber surgically placed in the peritoneal cavity. The chamber consists of a polypropylene cylinder with semipermeable membranes covering the chamber ends.

Diffusion of peritoneal fluid into and out of the chamber provides nutrients for the microbes. The progression of the "infection" may be followed by examining growth, the exoprotein production or RNA messages. The time experiments are done by sampling multiple chambers.

5                             (iii) *Endocarditis Model*

For bacteria, an important animal model effective in assessing pathogenicity and virulence is the endocarditis model (J. Santoro and M. E. Levinson, 1978, Infect. Immun. 19: 915-918). A rat endocarditis model can be used to assess colonization, virulence and proliferation.

10                           (iv) *Osteomyelitis Model*

A fourth model useful in the evaluation of pathogenesis is the osteomyelitis model (Spagnolo et al., 1993, Infect. Immun. 61: 5225-5230). Rabbits are used for these experiments. Anesthetized animals have a small segment of the tibia removed and microorganisms are microinjected into the wound. The excised bone segment is replaced 15 and the progression of the disease is monitored. Clinical signs, particularly inflammation and swelling are monitored. Termination of the experiment allows histologic and pathologic examination of the infection site to complement the assessment procedure.

(v) *Murine Septic Arthritis Model*

A fifth model relevant to the study of microbial pathogenesis is a murine septic 20 arthritis model (Abdelnour et al., 1993, Infect. Immun. 61: 3879-3885). In this model mice are infected intravenously and pathogenic organisms are found to cause inflammation in distal limb joints. Monitoring of the inflammation and comparison of inflammation vs. inocula allows assessment of the virulence of related strains.

(vi) *Bacterial Peritonitis Model*

Finally, bacterial peritonitis offers rapid and predictive data on the virulence of 25 strains (M. G. Bergeron, 1978, Scand. J. Infect. Dis. Suppl. 14: 189-206; S. D. Davis, 1975, Antimicrob. Agents Chemother. 8: 50-53). Peritonitis in rodents, such as mice, can provide essential data on the importance of targets. The end point may be lethality or clinical signs can be monitored. Variation in infection dose in comparison to outcome allows evaluation 30 of the virulence of individual strains.

A variety of other *in vivo* models are available and may be used when appropriate for specific pathogens or specific test agents. For example, target organ recovery assays (Gordee et al., 1984, J. Antibiotics 37:1054-1065; Bannatyne et al., 1992, Infect. 20:168-

170) may be useful for fungi and for bacterial pathogens which are not acutely virulent to animals.

It is also relevant to note that the species of animal used for an infection model, and the specific genetic make-up of that animal, may contribute to the effective evaluation of  
5 the effects of a particular test agent. For example, immuno-incompetent animals may, in some instances, be preferable to immuno-competent animals. For example, the action of a competent immune system may, to some degree, mask the effects of the test agent as compared to a similar infection in an immuno-incompetent animal. In addition, many opportunistic infections, in fact, occur in immuno-compromised patients, so modeling an  
10 infection in a similar immunological environment is appropriate.

#### *10. Vaccines*

There are provided by the invention, products, compositions and methods for raising immunological response against a pathogen, especially *P. aeruginosa*. In one aspect, a  
15 polypeptide of the invention or a nucleic acid of the invention, or an antigenic fragment thereof, may be administered to a subject, optionally with a booster, adjuvant, or other composition that stimulates immune responses.

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal which comprises inoculating the  
20 individual with a polypeptide of the invention and/or a nucleic acid of the invention, adequate to produce antibody and/or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *P. aeruginosa* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing  
25 immunological response in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of a polypeptide of the invention and/or a nucleic acid of the invention *in vivo* in order to induce an immunological response, such as, to produce antibody and/or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual,  
30 preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise

DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual to a nucleic acid of the invention and/or a polypeptide encoded therefrom, wherein the composition comprises a recombinant nucleic acid of the invention and/or polypeptide encoded therefrom and/or comprises DNA and/or RNA which encodes and expresses an antigen of said nucleic acid of the invention, polypeptide encoded therefrom, or other polypeptide of the invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+T cells.

In another embodiment, the invention relates to compositions comprising a polypeptide of the invention and an adjuvant. The adjuvant can be any vehicle which would typically enhance the antigenicity of a polypeptide, e.g., minerals (for instance, alum, aluminum hydroxide or aluminum phosphate), saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria in mineral oil, Freund's complete adjuvant, bacterial products, such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, liposomes, or any of the other adjuvants known in the art. A polypeptide of the invention can be emulsified with, absorbed onto, or coupled with the adjuvant.

A polypeptide of the invention may be fused with co-protein or chemical moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, may further comprise an antigenic co-protein, such as lipoprotein D from *Hemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of a polypeptide of the invention.

Provided by this invention are compositions, particularly vaccine compositions, and methods comprising the polypeptides and/or polynucleotides of the invention and immunostimulatory DNA sequences, such as those described in Sato, Y. et al. *Science* 273: 352 (1996).

5       Also, provided by this invention are methods using the described polynucleotide or particular fragments thereof, which have been shown to encode non-variable regions of bacterial cell surface proteins, in polynucleotide constructs used in such genetic immunization experiments in animal models of infection with *P. aeruginosa*. Such experiments will be particularly useful for identifying protein epitopes able to provoke a prophylactic or therapeutic immune response. It is believed that this approach will allow for the subsequent preparation of monoclonal antibodies of particular value, derived from the requisite organ of the animal successfully resisting or clearing infection, for the development of prophylactic agents or therapeutic treatments of bacterial infection, particularly *P. aeruginosa* infection, in mammals, particularly humans.

10      15     A polypeptide of the invention may be used as an antigen for vaccination of a host to produce specific antibodies which protect against invasion of bacteria, for example by blocking adherence of bacteria to damaged tissue.

### *11. Array Analysis*

20      In part, the present invention is directed to the use of subject nucleic acids in arrays to assess gene expression. In another part, the present invention is directed to the use of subject nucleic acids in arrays for *P. aeruginosa*. In yet another part, the present invention contemplates using the subject nucleic acids to interact with probes contained on arrays.

25      In one aspect, the present invention contemplates an array comprising a substrate having a plurality of addresses, wherein at least one of the addresses has disposed thereon a capture probe that can specifically bind to a nucleic acid of the invention. In another aspect, the present invention contemplates a method for detecting expression of a nucleotide sequence which encodes a polypeptide of the invention, or a fragment thereof, using the foregoing array by: (a) providing a sample comprising at least one mRNA molecule; (b) exposing the sample to the array under conditions which promote hybridization between the capture probe disposed on the array and a nucleic acid complementary thereto; and (c) detecting hybridization between an mRNA molecule of the

sample and the capture probe disposed on the array, thereby detecting expression of a sequence which encodes for a polypeptide of the invention, or a fragment thereof.

Arrays are often divided into microarrays and macroarrays, where microarrays have a much higher density of individual probe species per area. Microarrays may have as many 5 as 1000 or more different probes in a 1 cm<sup>2</sup> area. There is no concrete cut-off to demarcate the difference between micro- and macroarrays, and both types of arrays are contemplated for use with the invention.

Microarrays are known in the art and generally consist of a surface to which probes 10 that correspond in sequence to gene products (e.g., cDNAs, mRNAs, oligonucleotides) are bound at known positions. In one embodiment, the microarray is an array (e.g., a matrix) in which each position represents a discrete binding site for a product encoded by a gene (e.g., a protein or RNA), and in which binding sites are present for products of most or almost all 15 of the genes in the organism's genome. In certain embodiments, the binding site or site is a nucleic acid or nucleic acid analogue to which a particular cognate cDNA can specifically hybridize. The nucleic acid or analogue of the binding site may be, e.g., a synthetic oligomer, a full-length cDNA, a less-than full length cDNA, or a gene fragment.

Although in certain embodiments the microarray contains binding sites for products 20 of all or almost all genes in the target organism's genome, such comprehensiveness is not necessarily required. Usually the microarray will have binding sites corresponding to at least 100, 500, 1000, 4000 genes or more. In certain embodiments, arrays will have anywhere from about 50, 60, 70, 80, 90, or even more than 95% of the genes of a particular organism represented. The microarray typically has binding sites for genes relevant to testing and confirming a biological network model of interest. Several exemplary human microarrays are publicly available.

25 The probes to be affixed to the arrays are typically polynucleotides. These DNAs can be obtained by, e.g., polymerase chain reaction (PCR) amplification of gene segments from genomic DNA, cDNA (e.g., by RT-PCR), or cloned sequences. PCR primers are chosen, based on the known sequence of the genes or cDNA, that result in amplification of unique fragments (e.g., fragments that do not share more than 10 bases of contiguous 30 identical sequence with any other fragment on the microarray). Computer programs are useful in the design of primers with the required specificity and optimal amplification properties. See, e.g., Oligo pl version 5.0 (National Biosciences). In an alternative embodiment, the binding (hybridization) sites are made from plasmid or phage clones of

genes, cDNAs (e.g., expressed sequence tags), or inserts therefrom (Nguyen et al., 1995, Genomics 29:207-209).

A number of methods are known in the art for affixing the nucleic acids or analogues to a solid support that makes up the array (Schena et al., 1995, Science 270:467-5 470; DeRisi et al., 1996, Nature Genetics 14:457-460; Shalon et al., 1996, Genome Res. 6:639-645; and Schena et al., 1995, Proc. Natl. Acad. Sci. USA 93:10539-11286).

Another method for making microarrays is by making high-density oligonucleotide arrays (Fodor et al., 1991, Science 251:767-773; Pease et al., 1994, Proc. Natl. Acad. Sci. USA 91:5022-5026; Lockhart et al., 1996, Nature Biotech 14:1675; U.S. Pat. Nos. 10 5,578,832; 5,556,752; and 5,510,270; Blanchard et al., 1996, 11: 687-90).

Other methods for making microarrays, e.g., by masking (Maskos and Southern, 1992, Nuc. Acids Res. 20:1679-1684), may also be used. In principal, any type of array, for example, dot blots on a nylon hybridization membrane (see Sambrook et al., Molecular Cloning - A Laboratory Manual (2nd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold 15 Spring Harbor, N.Y., 1989), could be used, although, as will be recognized by those of skill in the art.

The nucleic acids to be contacted with the microarray may be prepared in a variety of ways, and may include nucleotides of the subject invention. Such nucleic acids are often labeled fluorescently. Nucleic acid hybridization and wash conditions are chosen so that 20 the population of labeled nucleic acids will specifically hybridize to appropriate, complementary nucleic acids affixed to the matrix. Non-specific binding of the labeled nucleic acids to the array can be decreased by treating the array with a large quantity of non-specific DNA -- a so-called "blocking" step.

When fluorescently labeled probes are used, the fluorescence emissions at each site 25 of a transcript array may be detected by scanning confocal laser microscopy. When two fluorophores are used, a separate scan, using the appropriate excitation line, is carried out for each of the two fluorophores used. Fluorescent microarray scanners are commercially available from Affymetrix, Packard BioChip Technologies, BioRobotics and many other suppliers. Signals are recorded, quantitated and analyzed using a variety of computer 30 software.

According to the method of the invention, the relative abundance of an mRNA in two cells or cell lines is scored as a perturbation and its magnitude determined (i.e., the abundance is different in the two sources of mRNA tested), or as not perturbed (i.e., the

relative abundance is the same). As used herein, a difference between the two sources of RNA of at least a factor of about 25% (RNA from one source is 25% more abundant in one source than the other source), more usually about 50%, even more often by a factor of about 2 (twice as abundant), 3 (three times as abundant) or 5 (five times as abundant) is scored as  
5 a perturbation. Present detection methods allow reliable detection of difference of an order of about 2-fold to about 5-fold, but more sensitive methods are expected to be developed.

In addition to identifying a perturbation as positive or negative, it is advantageous to determine the magnitude of the perturbation. This can be carried out, as noted above, by calculating the ratio of the emission of the two fluorophores used for differential labeling,  
10 or by analogous methods that will be readily apparent to those of skill in the art.

In certain embodiments, the data obtained from such experiments reflects the relative expression of each gene represented in the microarray. Expression levels in different samples and conditions may now be compared using a variety of statistical methods.

15

#### *12. Pharmaceutical Compositions*

Pharmaceutical compositions of this invention include any modulator identified according to the present invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier, adjuvant, or vehicle. The term "pharmaceutically acceptable carrier" refers to a carrier(s) that is "acceptable" in the sense of being compatible with the other ingredients of a composition and not deleterious to the recipient thereof.  
20

Methods of making and using such pharmaceutical compositions are also included in the invention. The pharmaceutical compositions of the invention can be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally, or  
25 via an implanted reservoir. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra articular, intrasynovial, intrasternal, intrathecal, intralesional, and intracranial injection or infusion techniques.

Dosage levels of between about 0.01 and about 100 mg/kg body weight per day, preferably between about 0.5 and about 75 mg/kg body weight per day of the modulators  
30 described herein are useful for the prevention and treatment of disease and conditions, including *P. aeruginosa* mediated diseases and conditions. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. A typical

preparation will contain from about 5% to about 95% active compound (w/w). Alternatively, such preparations contain from about 20% to about 80% active compound.

### *13. Antimicrobial Agents*

5       The polypeptides of the invention may be used to develop antimicrobial agents for use in a wide variety of applications. The uses are as varied as surface disinfectants, topical pharmaceuticals, personal hygiene applications (e.g., antimicrobial soap, deodorant or the like), additives to cell culture medium, and systemic pharmaceutical products. 10      Antimicrobial agents of the invention may be incorporated into a wide variety of products and used to treat an already existing microbial infection/contamination or may be used prophylactically to suppress future infection/contamination.

The antimicrobial agents of the invention may be administered to a site, or potential site, of infection/contamination in either a liquid or solid form. Alternatively, the agent may be applied as a coating to a surface of an object where microbial growth is undesirable 15     using nonspecific absorption or covalent attachment. For example, implants or devices (such as linens, cloth, plastics, heart pacemakers, surgical stents, catheters, gastric tubes, endotracheal tubes, prosthetic devices) can be coated with the antimicrobials to minimize adherence or persistence of bacteria during storage and use. The antimicrobials may also be incorporated into such devices to provide slow release of the agent locally for several 20     weeks during healing. The antimicrobial agents may also be used in association with devices such as ventilators, water reservoirs, air-conditioning units, filters, paints, or other substances. Antimicrobials of the invention may also be given orally or systemically after transplantation, bone replacement, during dental procedures, or during implantation to prevent colonization with bacteria.

25      In another embodiment, antimicrobial agents of the invention may be used as a food preservative or in treating food products to eliminate potential pathogens. The latter use might be targeted to the fish and poultry industries that have serious problems with enteric pathogens which cause severe human disease. In a further embodiment, the agents of the invention may be used as antimicrobials for food crops, either as agents to reduce post 30     harvest spoilage or to enhance host resistance. The antimicrobials may also be used as preservatives in processed foods either alone or in combination with antibacterial food additives such as lysozymes.

In another embodiment, the antimicrobials of the invention may be used as an additive to culture medium to prevent or eliminate infection of cultured cells with a pathogen.

## 5 EXEMPLIFICATION

The invention now being generally described, it will be more readily understood by reference to the following examples which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention in any way.

### 10 *EXAMPLE 1 Isolation and Cloning of Nucleic Acid*

*Pseudomonas aeruginosa* is an opportunistic Gram-negative bacilli found in sewage, plants, and sometimes the intestine. It is capable of infecting various organs and has been identified in numerous infections including those in the ears, lungs, urinary tract, blood and in burns and surgical wound infections. Polynucleotide sequences were obtained 15 from The Institute of Genomic Research (TIGR) (Rockville, MD; [www.tigr.org](http://www.tigr.org)). Chromosomal DNA was acquired from the American Type Culture Collection (ATCC; reference #17933D).

20 The coding sequences of the subject nucleic acid sequences (predicted) are obtained by reference to either publicly available databases or from the use of a bioinformatics program that is used to select the coding sequence of interest from the applicable genome. For example, coding sequences for the genome of *P. aeruginosa* may be obtained from NCBI (<http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/framik?db=Genome&gi=163>).

25 The coding DNA is amplified from purified genomic DNA using PCR with primers that are identified with a computer program. The PCR primers are selected so as to introduce restriction enzyme cleavage sites at the flanking regions of the DNA (e.g., NdeI and BglII). The forward and reverse primers have SEQ ID NO: 5 and SEQ ID NO: 6. The sequences of the primers are shown in FIGURE 5, and their respective restriction sites and melting temperatures are shown in Table 1 of FIGURE 6.

30 The PCR reaction is performed using 50-100 ng of chromosomal DNA and 2 Units of a high fidelity DNA Polymerase (for example *Pfu* Turbo (Stratagene) or Platinum *Pfx* (Invitrogen)). The thermocycling conditions for the PCR process include a DNA melting step at 94°C for 45 sec, a primer annealing step at 48°C - 58°C (depending on Primer [Tm]) for 45 sec, and an extension step at 68°C - 72°C (depending on enzyme) for 1 min 45 sec -

2 min 30 sec (depending on size of DNA). After 25-30 cycles, a final blocking step at 72°C for 9 min is carried out.

The amplified nucleic acid product is isolated from the PCR cocktail using silica-gel membrane based column chromatography (Qiagen). The quality of the PCR product is 5 assessed by resolving an aliquot of amplified product on a 1% agarose gel. The DNA is quantified spectrophotometrically at A<sub>260</sub> or by visualizing the resolved genes with a 302 nm UV-B light source.

The PCR product is directionally cloned into the polylinker region of any of three expression vectors: pET28 (Novagen), pET15 (Novagen) or pGEX (Pharmacia/LKB Biotechnology). Additional restriction enzyme sites may be engineered into the 10 expression vectors to allow for simultaneous clones to be prepared having different purification tags. After the ligation reaction, the DNA is transformed into competent *E. coli* cells (Strains XL1-Blue (Stratagene) or DH5 (Invitrogen)) via heat shock or electroporation as described in Sambrook, et al., Molecular Cloning: A Laboratory Manual, 15 2<sup>nd</sup> Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). The expression vectors contain the bacteriophage T7 promoter for RNA polymerase, and the *E. coli* strain used produces T7 RNA polymerase upon induction with isopropyl-β-D-thiogalactoside (IPTG). The sequence of the cloning site adds a Glutathione S-transferase (GST) tag, or a polyhistidine (6X His) tag, at the N- or C- terminus of the recombinant 20 protein. The cloning site also inserts a cleavage site for the thrombin or Tev (Invitrogen) enzymes between the recombinant protein and the N- or C-terminal GST or polyhistidine tag.

Transformants are selected using the appropriate antibiotic (Ampicillin or Kanamycin) and identified using PCR, or another method, to analyze their DNA. The 25 polynucleotide sequence cloned into the expression construct is then isolated using a modified alkaline lysis method (Birnboim, H.C., and Doly, J. (1979) *Nucl. Acids Res.* 7, 1513-1522.) The sequence of the clone is verified by standard polynucleotide sequencing methods. The published nucleic acid and amino acid sequences are presented in FIGURE 1 and FIGURE 2. The experimentally determined nucleic acid sequence is presented in 30 FIGURE 3, and the amino acid sequence predicted from the sequence of FIGURE 3 is presented in FIGURE 4.

The expression construct is transformed into a bacterial host strain BL21-Gold (DE3) supplemented with a plasmid called pUBS520, which directs expression of tRNA for

arginine (agg and aga) and serves to augment the expression of the recombinant protein in the host cell (Gene, vol. 85 (1989) 109-114). The expression construct may also be transformed into BL21-Gold (DE3) without pUBS520, BL21-Gold (DE3) Codon-Plus (RIL) or (RP) (Stratagene) or Roseatta (DE3) (Novagen), the latter two of which contain genes encoding tRNAs. Alternatively, the expression construct may be transformed into BL21 STAR *E. coli* (Invitrogen) cells which has an RNase deficiency that reduces degradation of recombinant mRNA transcript and therefore increases the protein yield. The recombinant protein is then assayed for positive overexpression in the host and the presence of the protein in the cytoplasmic (water soluble) region of the cell.

10

***EXAMPLE 2 Test Protein Expression and Solubility***

(a) Test Expression

Transformed cells are grown in LB medium supplemented with the appropriate antibiotics up to a final concentration of 100 µg/ml. The cultures are shaken at 37°C until they reach an optical density ( $OD_{600}$ ) between 0.6 and 0.7. The cultures are then induced with isopropyl-beta-D-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM at 15°C for 10 hours, 25°C for 4 hours, or 30°C for 4 hours.

(b) Method One for Determining Protein Solubility Levels

The cells are harvested by centrifugation and subjected to a freeze/thaw cycle. The cells are lysed using detergent, sonication, or incubation with lysozyme. Total and soluble proteins are assayed using a 26-well BioRad Criterion gel running system. The proteins are stained with an appropriate dye (Coomassie, Silver stain, or Sypro-Red) and visualized with the appropriate visualization system. Typically, recombinant protein is seen as a prominent band in the lanes of the gel representing the soluble fraction.

(c) Method Two for Determining Protein Solubility Levels

The soluble and insoluble fractions (in the presence of 6M urea) of the cell pellet are bound to the appropriate affinity column. The purified proteins from both fractions are analysed by SDS-PAGE and the levels of protein in the soluble fraction are determined.

The approximate percent solubility of the polypeptide having the sequence of SEQ ID NO: 4 is determined using one of the foregoing methods, and the resulting percent solubility is presented in Table 1 of FIGURE 6.

*EXAMPLE 3 Native Protein Expression*

The expression construct clone encoding the soluble polypeptide having the amino acid sequence of SEQ ID NO: 4 is introduced into an expression host. The resultant cell line is then grown in culture. The method of growth is dependant on whether the protein to be purified is a native protein or a labeled protein. For native and  $^{15}\text{N}$  labeled protein production, a Gold-pUBS520 (as described above), BL21-Gold (DE3) Codon-Plus (RIL) or (RP), or BL21 STAR *E. Coli* cell line is used. For generating proteins metabolically labeled with selenium, the clone is introduced into a strain called B834 (Novagen). The methods for expressing labeled polypeptides of the invention are described in the Examples that follow.

In one method for expressing an unlabeled polypeptide of the invention, 2L LB cultures or 1L TB cultures are inoculated with a 1% (v/v) starter culture ( $\text{OD}_{600}$  of 0.8). The cultures are shaken at 37°C and 200 rpm and grown to an  $\text{OD}_{600}$  of 0.6-0.8 followed by induction with 0.5mM IPTG at 15°C and 200 rpm for at least 10 hours or at 25°C for 4 hours.

The cells are harvested by centrifugation and the pellets are resuspended in 25 ml HEPES buffer (50 mM, pH 7.5), supplemented with 100 $\mu\text{l}$  of protease inhibitors (PMSF and benzamidine (Sigma)) and flash-frozen in liquid nitrogen.

Alternatively, for an unlabeled polypeptide of the invention, a starter culture is prepared in a 300 mL Tunair flask (Shelton Scientific) by adding 20 mL of medium having 47.6 g/L of Terrific Broth and 1.5% glycerol in dH<sub>2</sub>O followed by autoclaving for 30 minutes at 121°C and 15 psi. When the broth cools to room temperature, the medium is supplemented with 6.3  $\mu\text{M}$  CoCl<sub>2</sub>-6H<sub>2</sub>O, 33.2  $\mu\text{M}$  MnSO<sub>4</sub>-5H<sub>2</sub>O, 5.9  $\mu\text{M}$  CuCl<sub>2</sub>-2H<sub>2</sub>O, 8.1  $\mu\text{M}$  H<sub>3</sub>BO<sub>3</sub>, 8.3  $\mu\text{M}$  Na<sub>2</sub>MoO<sub>4</sub>-2H<sub>2</sub>O, 7  $\mu\text{M}$  ZnSO<sub>4</sub>-7H<sub>2</sub>O, 108  $\mu\text{M}$  FeSO<sub>4</sub>-7H<sub>2</sub>O, 68  $\mu\text{M}$  CaCl<sub>2</sub>-2H<sub>2</sub>O, 4.1  $\mu\text{M}$  AlCl<sub>3</sub>-6H<sub>2</sub>O, 8.4  $\mu\text{M}$  NiCl<sub>2</sub>-6H<sub>2</sub>O, 1 mM MgSO<sub>4</sub>, 0.5% v/v of Kao and Michayluk vitamins mix (Sigma; Cat. No. K3129), 25  $\mu\text{g}/\text{mL}$  Carbenicillin, and 50  $\mu\text{g}/\text{mL}$  Kanamycin. The medium is then inoculated with several colonies of the freshly transformed expression construct of interest. The culture is incubated at 37°C and 260 rpm for about 3 hours and then transferred to a 2.5L Tunair Flask containing 1L of the above media. The 1L culture is then incubated at 37°C with shaking at 230-250 rpm on an orbital shaker having a 1 inch orbital diameter. When the culture reaches an  $\text{OD}_{600}$  of 3-6 it is induced with 0.5 mM IPTG. The induced culture is then incubated at 15°C with shaking at

230-250 rpm or faster for about 6-15 hours. The cells are harvested by centrifugation at 3500 rpm at 4°C for 20 minutes and the cell pellet is resuspended in 15 mL ice cold binding buffer (Hepes 50 mM, pH 7.5) and 100 µl of protease inhibitors (50 mM PMSF and 100 mM Benzamidine, stock concentration) and flash frozen.

5

*EXAMPLE 4 Expression of Selmet Labeled Polypeptides*

The freshly transformed cell, harboring a plasmid with a nucleic acid encoding a polypeptide of the invention, is inoculated into 20 ml of NMM (New Minimal Medium) and shaken at 37°C for 8-9 hours. This culture is then transferred into a 6L Erlenmeyer flask containing 2L of minimum medium (M9). The media is supplemented with all amino acids except methionine. All amino acids are added as a solution except for Tyrosine, Tryptophan and Phenylalanine which are added to the media in powder format. As well the media is supplemented with MgSO<sub>4</sub> (2mM final concentration), FeSO<sub>4</sub>.7H<sub>2</sub>O (25mg/L final concentration), Glucose (0.4% final concentration), CaCl<sub>2</sub> (0.1mM final concentration) and 10 Seleno-L-Methionine (40mg/L final concentration). When the OD<sub>600</sub> of the cell culture reaches 0.8-0.9, IPTG (0.4 mM final concentration) is added to the medium for protein induction, and the cell culture is kept shaking at 15°C for 10 hours. The cells are harvested by centrifugation at 3500 rpm at 4°C for 20 minutes and the cell pellet is resuspended in 15 mL cold binding buffer (Hepes 50 mM, pH 7.5) and 100 µl of protease inhibitors (PMSF and Benzamidine) and flash frozen.

Alternatively, a starter culture is prepared in a 300 mL Tunair flask (Shelton Scientific) by adding 50 mL of sterile medium having 10% 10XM9 (37.4 mM NH<sub>4</sub>Cl (Sigma; Cat. No. A4514), 44 mM KH<sub>2</sub>PO<sub>4</sub> (Bioshop, Ontario, Canada; Cat. No. PPM 302), 96 mM Na<sub>2</sub>HPO<sub>4</sub> (Sigma; Cat. No. S2429256), and 96 mM Na<sub>2</sub>HPO<sub>4</sub>.7H<sub>2</sub>O (Sigma; Cat. No. S9390) final concentration), 450 µM alanine, 190 µM arginine, 302 µM asparagine, 300 µM aspartic acid, 330 µM cysteine, 272 µM glutamic acid, 274 µM glutamine, 533 µM glycine, 191 µM histidine, 305 µM isoleucine, 305 µM leucine, 220 µM lysine, 242 µM phenylalanine, 348 µM proline, 380 µM serine, 336 µM threonine, 196 µM tryptophan, 220 µM tyrosine, and 342 µM valine, 204 µM Seleno-L-Methionine (Sigma; Cat. No. S3132), 25 0.5% v/v of Kao and Michayluk vitamins mix (Sigma; Cat. No. K3129), 2 mM MgSO<sub>4</sub> (Sigma; Cat. No. M7774), 90 µM FeSO<sub>4</sub>.7H<sub>2</sub>O (Sigma; Cat. No. F8633), 0.4% glucose (Sigma; Cat. No. G-5400), 100 µM CaCl<sub>2</sub> (Bioshop, Ontario, Canada; Cat. No. CCL 302), 30

50 µg/mL Ampicillin, and 50 µg/mL Kanamycin in dH<sub>2</sub>O. The medium is then inoculated with several colonies of *E. coli* B834 cells (Novagen) freshly transformed with an expression construct clone encoding the polypeptide of interest. The culture is then incubated at 37°C and 200 rpm until it reaches an OD<sub>600</sub> of ~1 and is then transferred to a  
5 2.5L Tunair Flask containing 1L of the above media. The 1L culture is incubated at 37°C with shaking at 200 rpm until the culture reaches an OD<sub>600</sub> of 0.6-0.8 and is then induced with 0.5 mM IPTG. The induced culture is incubated overnight at 15°C with shaking at 200 rpm. The cells are harvested by centrifugation at 4200 rpm at 4°C for 20 minutes and the cell pellet is resuspended in 15 mL ice cold binding buffer (Hepes 50 mM, pH 7.5) and  
10 100 µl of protease inhibitors (50 mM PMSF and 100 mM Benzamidine, stock concentration) and flash frozen.

Alternatively, the cell harboring a plasmid with a nucleic acid encoding a polypeptide of the invention is inoculated into 10 ml of M9 minimum medium and kept shaking at 37°C for 8-9 hours. This culture is then transferred into a 2L Baffled Flask  
15 (Corning) containing 1L minimum medium. The media is supplemented with all amino acids except methionine. All are added as a solution, except for Phenylalanine, Alanine, Valine, Leucine, Isoleucine, Proline, and Tryptophan which are added to the media in powder format. As well the media is supplemented with MgSO<sub>4</sub> (2mM final concentration), FeSO<sub>4</sub>·7H<sub>2</sub>O (25 mg/L final concentration), Glucose (0.5% final concentration), CaCl<sub>2</sub> (0.1  
20 mM final concentration) and Seleno-Methionine (50 mg/L final concentration). When the OD<sub>600</sub> of the cell culture reaches 0.8-0.9, IPTG (0.8 mM final concentration) is added to the medium for protein induction, and the cell culture is kept shaking at 25°C for 4 hours. The cells are harvested by centrifuged at 3500 rpm at 4°C for 20 minutes and the cell pellet is resuspended in 10 mL cold binding buffer (Hepes 50 mM, pH 7.5) and 100 µl of protease  
25 inhibitors (PMSF and Benzamidine) and flash frozen.

#### *EXAMPLE 5 Expression of <sup>15</sup>N Labeled Polypeptides*

The cell, harboring a plasmid with a nucleic acid encoding a polypeptide of the invention, is inoculated into 2L of minimal media (containing <sup>15</sup>N isotope, Cambridge Isotope Lab) in a 6L Erlenmeyer flask. The minimal media is supplemented with 0.01 mM ZnSO<sub>4</sub>, 0.1 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, 5 mg/L Thiamine.HCl, and 0.4% glucose. The 2L culture is grown at 37°C and 200 rpm to an OD<sub>600</sub> of between 0.7-0.8. The culture is then

induced with 0.5 mM IPTG and allowed to shake at 15°C for 14 hours. The cells are harvested by centrifugation and the cell pellet is resuspended in 15 mL cold binding buffer and 100 µl of protease inhibitor and flash frozen. The protein is then purified as described below.

5        Alternatively, the freshly transformed cell, harboring a plasmid with the gene of interest, is inoculated into 10 mL of M9 media (with  $^{15}\text{N}$  isotope) and supplemented with 0.01 mM ZnSO<sub>4</sub>, 0.1 mM CaCl<sub>2</sub>, 1 mM MgSO<sub>4</sub>, 5 mg/L Thiamine.HCl, and 0.4% glucose. After 8-10 hours of growth at 37°C, the culture is transferred to a 2L Baffled flask (Corning) containing 990 mL of the same media. When OD<sub>600</sub> of the culture is between  
10      0.7-0.8, protein production is initiated by adding IPTG to a final concentration of 0.8 mM and lowering the temperature to 25°C. After 4 hours of incubation at this temperature, the cells are harvested, and the cell pellet is resuspended in 10 mL cold binding buffer (Hepes 50 mM, pH 7.5) and 100 µl of protease inhibitor and flash frozen.

15      *EXAMPLE 6 Method One for Purifying Polypeptides of the Invention*

The frozen pellets are thawed and sonicated to lyse the cells (5 x 30 seconds, output 4 to 5, 80% duty cycle, in a Branson Sonifier, VWR). The lysates are clarified by centrifugation at 14,000 rpm for 60 min at 4°C to remove insoluble cellular debris. The supernatants are removed and supplemented with 1 µl of Benzonase Nuclease (25 U/µl, Novagen).

The recombinant protein is purified using DE52 (anion exchanger, Whatman) and Ni-NTA columns (Qiagen). The DE52 columns (30 mm wide, Biorad) are prepared by mixing 10 grams of DE52 resin in 25 ml of 2.5 M NaCl per protein sample, applying the resin to the column and equilibrating with 30 ml of binding buffer (50 mM in HEPES, pH 7.5, 5% glycerol (v/v), 0.5 M NaCl, 5 mM imidazole). Ni-NTA columns are prepared by adding 3.5-8 ml of resin to the column (20 mm wide, Biorad) based on the level of expression of the recombinant protein and equilibrating the column with 30 ml of binding buffer. The columns are arranged in tandem so that the protein sample is first passed over the DE52 column and then loaded directly onto the Ni-NTA column.

25      The Ni-NTA columns are washed with at least 150 ml of wash buffer (50mM HEPES, pH 7.5, 5% glycerol (v/v), 0.5 M NaCl, 30 mM imidazole) per column. A pump may be used to load and/or wash the columns. The protein is eluted off of the Ni-NTA

column using elution buffer (50 mM in HEPES, pH 7.5, 5% glycerol (v/v), 0.5 M NaCl, 250 mM imidazole) until no more protein is observed in the aliquots of eluate as measured using Bradford reagent (Biorad). The eluate is supplemented with 1 mM of EDTA and 0.2 mM DTT.

5       The samples are assayed by SDS-PAGE and stained with Coomassie Blue, with protein purity determined by visual staining.

Two methods may be used to remove the His tag located at either the C or N-terminus. In certain instances, the His tag may not be removed. In either case, the expressed polypeptide will have additional residues attributable to the His tag, as shown in  
10 the following table:

<i>SEQ ID NO for Additional Residues</i>	<i>Additional Residues</i>	<i>Type of Tag and Whether or Not Removed</i>
N/A	GSH	His tag removed from N-terminus
SEQ ID NO: 7	MGSSHHHHHHSSGLVPRGSH	His tag not removed from N-terminus
SEQ ID NO: 8	GSENLYFQGHHHHHH	His tag removed from C-terminus
SEQ ID NO: 9	GSENLYFQ	His tag not removed from C-terminus

In method one, a sample of purified polypeptide is supplemented with 2.5 mM CaCl<sub>2</sub> and an appropriate amount of thrombin (the amount added will vary depending on the activity of the enzyme preparation) and incubated for ~20-30 minutes on ice in order to  
15 remove the His tag. In method two, a sample of purified polypeptide is combined with thirty units of recombinant TEV protease in 50 mmol TRIS HCl pH = 8.0, 0.5 mmol EDTA and 1 mmol DTT, followed by incubation at 4°C overnight, to remove the His tag.

The protein sample is then dialyzed in dialysis buffer (10mM HEPES, pH 7.5, 5% glycerol (v/v) and 0.5 M NaCl) for at least 8 hours using a Slide-A-Lyzer (Pierce)  
20 appropriate for the molecular weight of the recombinant protein. An aliquot of the cleaved and dialyzed samples is then assayed by SDS-PAGE and stained with Coomassie Blue to determine the purity of the protein and the success of cleavage.

The remainder of the sample is centrifuged at 2700 rpm at 4°C for 10-15 minutes to remove any precipitant and supplemented with 100 µl of protease inhibitor cocktail (0.1 M  
25 benzamidine and 0.05 M PMSF) (NO Bioshop). The protein is then applied to a second Ni-NTA column (~8 ml of resin) to remove the His-tags and eluted with binding buffer or

wash buffer until no more protein is eluting off the column as assayed using the Bradford reagent. The eluted sample is supplemented with 1 mM EDTA and 0.6 mM of DTT and concentrated to a final volume of ~15 mls using a Millipore Concentrator with an appropriately sized filter at 2700 rpm at 4°C. The samples are then dialyzed overnight  
5 against crystallization buffer and concentrated to final volume of 0.3-0.7 ml.

***EXAMPLE 7 Method Two for Purifying Polypeptides of the Invention***

The frozen pellets are thawed and supplemented with 100 µl of protease inhibitor (0.1 M benzamidine and 0.05 M PMSF), 0.5% CHAPS, and 4 U/ml Benzonase Nuclease.  
10 The sample is then gently rocked on a Nutator (VWR, setting 3) at room temperature for 30 minutes. The cells are then lysed by sonication (1 x 30 seconds, output 4 to 5, 80% duty cycle, in a Branson Sonifier, VWR) and an aliquot is saved for a gel sample.

The recombinant protein is purified using a three column system. The columns are set up in tandem so that the lysate flows from a Biorad Econo (5.0 x 30 cm x 589 ml)  
15 "lysate" column onto a Biorad Econo (2.5 x 20 cm x 98 ml) DE52 column and finally onto a Biorad Econo (1.5 x 15 cm x 27 ml) Ni-NTA column. The lysate is mixed with 10 g of equilibrated DE52 resin and diluted to a total volume of 300 ml with binding buffer. This mixture is poured into the first column which is empty. The remainder of the purification procedure is described in EXAMPLE 6 above.

20

***EXAMPLE 8 Method Three for Purifying Polypeptides of the Invention***

The frozen pellets are thawed and sonicated to lyse the cells (5 x 30 seconds, output 4 to 5, 80% duty cycle, in a Branson Sonifier, VWR). The lysates are clarified by centrifugation at 14000 rpm for 60 min at 4°C to remove insoluble cellular debris. The  
25 supernatants are removed and supplemented with 1 µl of Benzonase Nuclease (25 U/µl, Novagen).

The recombinant protein is purified using DE52 (anion exchanger, Whatman) and Glutathione sepharose columns (Glutathione-Superflow resin, Clontech). The DE52 columns (30 mm wide, Biorad) are prepared by mixing 10 grams of DE52 resin in 20 ml of  
30 2.5 M NaCl per protein sample, applying the resin to the column and equilibrating with 30 ml of loading buffer (50mM in HEPES, pH 7.5, 10% glycerol (v/v), 0.5 M NaCl, 1 mM EDTA, 1 mM DTT). Glutathione sepharose columns are prepared by adding 3 ml of resin to the column (20 mm wide, Biorad) and equilibrating the column with 30 ml of loading

buffer. The columns are arranged in tandem so that the protein sample is first passed over the DE52 column and then loads directly onto the Glutathione sepharose column.

The columns are washed with at least 150 ml of loading buffer supplemented with protease inhibitor cocktail (0.1 M benzamidine and 0.05 M PMSF) per column. A pump  
5 may be used to load and/or wash the columns. The protein is eluted off of the Glutathione sepharose column using elution buffer (20mM HEPES, pH 7.5, 0.5 M NaCl, 1 mM EDTA, 1 mM DTT; 25 mM glutathione (reduced form)) until no more protein is observed in the aliquots of eluate as measured using Biorad Bradford reagent.

The GST tag may be removed using thrombin or other procedures known in the art.

10 The protein samples are then dialyzed into crystallization buffer (10 mM Hepes, pH 7.5, 500 mM NaCl) to remove free glutathione and assayed by SDS-PAGE followed by staining with Coomassie blue. Prior to use or storage, the samples are concentrated to final volume of 0.3-0.5 ml.

Using one or more of the methods described above, purified polypeptide having  
15 SEQ ID NO: 4 is obtained in a yield of approximately 3.9 mg per liter of culture. The purified polypeptide is essentially the only protein visualized in the SDS-PAGE assay using Coomassie Blue described above, which is at least about 95% or greater purity. The polypeptide so expressed and purified has the following additional amino acid residues from the removed His tag at the N-terminus: GSH.

20 The protein samples so prepared and purified may be used in the biophysical studies that follow, with or without the His tag or the residual amino acids resulting from removal of the His tag. In certain instances, such as EXAMPLE 11, the polypeptide used may be a fusion protein with a specific tag.

A stable solution of purified polypeptide having SEQ ID NO: 4, prepared and  
25 purified as described above, may be prepared with 11.0 mg (or a lesser amount) of protein in one ml of either the dialysis or crystallization buffers (or possibly both) described above in EXAMPLE 6 or EXAMPLE 8, respectively.

Certain of the foregoing information is also set forth in Table 1 of FIGURE 6

30 **EXAMPLE 9 Mass Spectrometry Analysis via Fingerprint Mapping**

A gel slice from a purification protocol described above containing a polypeptide of the invention is cut into 1 mm cubes and 10 to 20  $\mu$ l of 1% acetic acid is added. After washing with 100 - 150  $\mu$ l HPLC grade water and removal of the liquid, acetonitrile (~200

μl, approximately 3 to 4 times the volume of the gel particles) is added followed by incubation at room temperature for 10 to 15 minutes with vortexing. A second acetonitrile wash may be required to completely dehydrate the gel particles. The protein in the gel particles is reduced at 50 degrees Celsius using 10 mM dithiothreitol (in 100 mM ammonium bicarbonate) and then alkylated at room temperature in the dark using 55 mM iodoacetamide (in 100 mM ammonium bicarbonate). The gel particles are rinsed with a minimal volume of 100 mM ammonium bicarbonate before a trypsin (50 mM ammonium bicarbonate, 5 mM CaCl<sub>2</sub>, and 12.5 ng/μl trypsin) solution is added. The gel particles are left on ice for 30 to 45 minutes (after 20 minutes incubation more trypsin solution is added).

5      10     The excess trypsin solution is removed and 10 to 15 μl digestion buffer without trypsin is added to ensure the gel particles remain hydrated during digestion. After digestion at 37°C, the supernatant is removed from the gel particles. The peptides are extracted from the gel particles with 2 changes of 100 μL of 100 mM ammonium bicarbonate with shaking for 45 minutes and pooled with the initial gel supernatant. The extracts are acidified to 1% (v/v)

10    15     with 100% acetic acid.

The tryptic peptides are purified with a C18 reverse phase resin. 250 μL of dry resin is washed twice with methanol and twice with 75% acetonitrile/1% acetic acid. A 5:1 slurry of solvent:resin is prepared with 75% acetonitrile/1% acetic acid. To the extracted peptides, 2 μL of the resin slurry is added and the solution is shaken for 30 minutes at room temperature. The supernatant is removed and replaced with 200 μL of 2% acetonitrile/1% acetic acid and shaken for 5-15 minutes. The supernatant is removed and the peptides are eluted from the resin with 15 μL of 75% acetonitrile/1% acetic acid with shaking for about 5 minutes. The peptide and slurry mixture is applied to a filter plate and centrifuged, and the filtrate is collected and stored at -70°C until use.

20    25     Alternatively, the tryptic peptides are purified using ZipTip<sub>C18</sub> (Millipore, Cat # ZTC18S960). The ZipTips are first pre-wetted by aspirating and dispensing 100% methanol. The tips are then washed with 2% acetonitrile/1% acetic acid (5 times), followed by 65% acetonitrile/1% acetic (5 times) and returned to 2% acetonitrile/1% acetic acid (10 times). The digested peptides are bound to the ZipTips by aspirating and dispensing the samples 5 times. Salts are removed by washing ZipTips with 2% acetonitrile/1% acetic acid (5 times). 10 μL of 65% acetonitrile/1% acetic acid is collected by the ZipTips and dispensed into a 96-well microtitre plate.

Analytical samples containing tryptic peptides are subjected to MALDI-TOF mass spectrometry. Samples are mixed 1:1 with a matrix of  $\alpha$ -cyano-4-hydroxy-*trans*-cinnamic acid. The sample/matrix mixture is spotted on to the MALDI sample plate with a robot, either a Gilson 215 liquid handler or BioMek FX laboratory automation workstation 5 (Beckman). The sample/matrix mixture is allowed to dry on the plate and is then introduced into the mass spectrometer. Analysis of the peptides in the mass spectrometer is conducted using both delayed extraction mode (400 ns delay) and an ion reflector to ensure high resolution of the peptides.

Internally-calibrated tryptic peptide masses are searched against databases using a 10 correlative mass matching algorithm. The Proteometrics software package (ProteoMetrics) is utilized for batch database searching of tryptic peptide mass spectra. Statistical analysis is performed on each protein match to determine its validity. Typical search constraints include error tolerances within 0.1 Da for monoisotopic peptide masses, carboxyamidomethylation of cysteines, no oxidation of methionines allowed, and 0 or 1 15 missed enzyme cleavages. The software calculates the probability that a candidate in the database search is the protein being analyzed, which is expressed as the Z-score. The Z-score is the distance to the population mean in unit of standard deviation and corresponds to the percentile of the search in the random match population. If a search is in the 95th percentile, for example, about 5% of random matches could yield a higher Z-score than the 20 search. A Z-score of 1.282 for a search indicates that the search is in the 90th percentile, a Z-score of 1.645 indicates that the search is in the 95th percentile, a Z-score of 2.326 indicates that the search is in the 99th percentile, and a Z-score of 3.090 indicates that the search is in the 99.9th percentile.

25           **EXAMPLE 10 Mass Spectrometry Analysis via High Mass**

A matrix solution of 25 mg/mL of 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid) in 66% (v/v) acetonitrile/1% (v/v) acetic acid is prepared along with an internal calibrant of carbonic anhydrase. On to a stainless steel polished MALDI target, 1.5  $\mu$ L of a protein solution (concentration of 2  $\mu$ g/ $\mu$ L) is spotted, followed immediately by 1.5 30  $\mu$ L of matrix. 3  $\mu$ L of 40% (v/v) acetonitrile/1% (v/v) acetic acid is then added to each spot has dried. The sample is either spotted manually or utilizing a Gilson 215 liquid handler or BioMek FX laboratory automation workstation (Beckman). The MALDI-TOF instrument

utilizes positive ion and linear detection modes. Spectra are acquired automatically over a mass to charge range from 0-150,000 Da, pulsed ion extraction delay is set at 200 ns, and 600 summed shots of 50-shot steps are completed.

The theoretical molecular weight of the protein for MALDI-TOF is determined  
5 from its amino acid sequence, taking into account any purification tag or residue thereof  
still present and any labels (e.g., selenomethionine or  $^{15}\text{N}$ ). To account for  $^{15}\text{N}$   
incorporation, an amount equal to the theoretical molecular weight of the protein divided by  
70 is added. The mass of water is subtracted from the overall molecular weight. The  
MALDI-TOF spectrum is calibrated with the internal calibrant of carbonic anhydrase  
10 (observed as either  $[\text{MH}^+_{\text{avg}}]$  29025 or  $[\text{MH}_2^{2+}]$  14513).

***EXAMPLE 11 Method One for Isolating and Identifying Interacting Proteins***

(a) Method One for Preparation of Affinity Column

Micro-columns are prepared using forceps to bend the ends of P200 pipette tips and  
15 adding 10  $\mu\text{l}$  of glass beads to act as a column frit. Six micro-columns are required for  
every polypeptide to be studied. The micro-columns are placed in a 96-well plate that has 1  
mL wells. Next, a series of solutions of the polypeptide having SEQ ID NO: 4 or other  
polypeptide of the invention, prepared and purified as described above and with a GST tag  
on either terminus, is prepared so as to give final amounts of 0, 0.1, 0.5, 1.0, and 2.0 mg of  
20 ligand per ml of resin volume.

A slurry of Glutathione-Sepharose 4B (Amersham) is prepared and 0.5 ml  
slurry/ligand is removed (enough for six 40- $\mu\text{g}$  aliquots of resin). Using a glass frit  
Buchner funnel, the resin is washed sequentially with three 10 ml portions each of distilled  
H<sub>2</sub>O and 1 M ACB (20 mM HEPES pH 7.9, 1 M NaCl, 10% glycerol, 1 mM DTT, and 1  
25 mM EDTA). The Glutathione-Sepharose 4B is completely drained of buffer, but not dried.  
The Glutathione-Sepharose 4B is resuspended as a 50% slurry in 1 M ACB and 80  $\mu\text{l}$  is  
added to each micro-column to obtain 40  $\mu\text{g}/\text{column}$ . The buffer containing the ligand  
concentration series is added to the columns and allowed to flow by gravity. The resin and  
ligand are allowed to cross-link overnight at 4°C. In the morning, micro-columns are  
30 washed with 100  $\mu\text{l}$  of 1 M ACB and allowed to flow by gravity. This is repeated twice  
more and the elutions are tested for cross-linking efficiency by measuring the amount of

unbound ligand. After washing, the micro-columns are equilibrated using 200 µl of 0.1 M ACB (20 mM HEPES pH 7.5, 0.1 M NaCl, 10% glycerol, 1 mM DTT, 1 mM EDTA).

In another method, the recombinant GST fusion protein can be replaced by a hexahistidine fusion peptide for use with NTA-Agarose (Qiagen) as the solid support. No 5 adaptation to the above protocol is required for the substitution of NTA agarose for GST Sepharose except that the recombinant protein requires a six histidine fusion peptide in place of the GST fusion.

15 (b) Method Two for Preparation of Affinity Column

In an alternative method, GST-Sepharose 4B may be replaced by Affi-gel 10 Gel 10 (Bio-Rad). The column resin for affinity chromatography could also be Affigel 10 resin which allows for covalent attachment of the protein ligand to the micro affinity column. An adaptation to the above protocol for the use of this resin is a pre-wash of the resin with 100% isopropanol. No fusion peptides or proteins are required for the use of Affigel 10 resin.

15 (c) Method One for Bacterial Extract Preparation

A *P. aeruginosa* extract is prepared from cell pellets using a French press followed by sonication. An *P. aeruginosa* cell pellet (~6 g) is suspended in 3 pellet volumes (~20 ml final volume) of 20 mM HEPES pH 7.5, 150 mM NaCl, 10% glycerol, 10 mM MgSO<sub>4</sub>, 10 mM CaCl<sub>2</sub>, 1 mM DTT, 1 mM PMSF, 1 mM benzamidine, 40 µg/ml RNase A, 75 units/ml 20 S1 nuclease, and 40 units/ml DNase 1. The cell suspension is lysed with one pass with a French Pressure Cell followed by sonication on ice using three bursts of 20 seconds each. The lysate is agitated at 4°C for 30 minutes, brought up to 0.5 M NaCl and then incubated for an additional 30 min at 4°C with agitation. The lysate is centrifuged at 25,000 rpm for 1 hr at 4°C in a Ti70 fixed angle Beckman rotor. The supernatant is removed and dialyzed 25 overnight in a 10,000 Mr dialysis membrane against dialysis buffer (20 mM HEPES pH 7.5, 10 % glycerol, 1 mM DTT, 1 mM EDTA, 100 mM NaCl, 10 mM MgSO<sub>4</sub>, 10 mM CaCl<sub>2</sub>, 1 mM benzamidine, and 1 mM PMSF). The dialyzed protein extract is removed from the dialysis tubing and frozen in one ml aliquots at -70°C.

30 (d) Method Two for Bacterial Extract Preparation

Bacterial cell extracts from *P. aeruginosa* are prepared from cell pellets using a Bead-Beater apparatus (Bio-spec Products Inc.) and zirconia beads (0.1 mm diameter). The bacterial cell pellet is suspended (~6 g) is suspended in 3 pellet volumes (~20 ml final

volume) of 20 mM HEPES pH 7.5, 150 mM NaCl, 10% glycerol, 10 mM MgSO<sub>4</sub>, 10 mM CaCl<sub>2</sub>, 1 mM DTT, 1 mM PMSF, 1 mM benzamidine, 40 µg/ml RNase A, 75 units/ml S1 nuclease, and 40 units/ml DNase 1. The cells are lysed with 10 pulses of 30 sec between 90 sec pauses at a temperature of -5 °C. The lysate is separated from the zirconia beads 5 using a standard column apparatus. The lysate is centrifuged at 20000 rpm (48000 x g) in a Beckman JA25.50 rotor. The supernatant is removed and dialyzed overnight at 4 °C against dialysis buffer (20 mM HEPES pH 7.5, 10 % glycerol, 1 mM DTT, 1 mM EDTA, 100 mM NaCl, 10 mM MgSO<sub>4</sub>, 10 mM CaCl<sub>2</sub>, 1 mM benzamidine, and 1 mM PMSF). The dialyzed protein extract is removed from the dialysis tubing and frozen in one ml aliquots at -70°C.

10 (e) HeLa Cell Extract Preparation

A HeLa cell extract is prepared in the presence of protease inhibitors. Approximately 30 g of Hela cells are submitted to a freeze/thaw cycle and then divided into two tubes. To each tube 20 ml of Buffer A (10 mM HEPES pH 7.9, 1.5 mM MgCl, 10 mM KCl, 0.5 mM DTT, 0.5 mM PMSF) and a protease inhibitor cocktail are added. The cell 15 suspension is homogenized with 10 strokes (2 x 5 strokes) to lyse the cells. Buffer B (15 ml per tube) is added (50 mM HEPES pH 7.9, 1.5 mM MgCl, 1.26 M NaCl, 0.5 mM DTT, 0.5 mM PMSF, 0.5 mM EDTA, 75% glycerol) to each tube followed by a second round of homogenization (2 x 5 strokes). The lysates are stirred on ice for 30 minutes followed by centrifugation 37,000 rpm for 3 hr at 4°C in a Ti70 fixed angle Beckman rotor. The 20 supernatant is removed and dialyzed overnight in a 10,000 Mr dialysis membrane against dialysis buffer (20 mM HEPES pH 7.9, 10% glycerol, 1 mM DTT, 1 mM EDTA, and 1 M NaCl. The dialyzed protein extract is removed from the dialysis tubing and frozen in one ml aliquots at -70°C.

25 (f) Affinity Chromatography

Cell extract is thawed and diluted to 5 mg/ml prior to loading 5 column volumes onto each micro-column. Each column is washed with 5 column volumes of 0.1 M ACB. This washing is repeated once. Each column is then washed with 5 column volumes of 0.1 M ACB containing 0.1% Triton X-100. The columns are eluted with 4 column volumes of 1% sodium dodecyl sulfate into a 96 well PCR plate. To each eluted fraction is added one- 30 tenth volume of 10-fold concentrated loading buffer for SDS-PAGE.

(g) Resolution of the Eluted Proteins and Detection of Bound Proteins

The components of the eluted samples are resolved on SDS-polyacrylamide gels containing 13.8% polyacrylamide using the Laemmli buffer system and stained with silver

nitrate. The bands containing the interacting protein are excised with a clean scalpel. The gel volume is kept to a minimum by cutting as close to the band as possible. The gel slice is placed into one well of a low protein binding, 96-well round-bottom plate. To the gel slices is added 20  $\mu$ l of 1% acetic acid.

5

*EXAMPLE 12 Method Two for Isolating and Identifying Interacting Proteins*

Interacting proteins may be isolated using immunoprecipitation. Naturally-occurring bacterial or eukaryotic cells are grown in defined growth conditions or the cells can be genetically manipulated with a protein expression vector. The protein expression vector is used to transiently transfet the cDNA of interest into eukaryotic or prokaryotic cells and the protein is expressed for up to 24 or 48 hours. The cells are harvested and washed three times in sterile 20 mM HEPES (pH7.4)/Hanks balanced salts solution (H/H). The cells are finally resuspended in culture media and incubated at 37°C for 4-8 hr.

The harvested cells may be subjected to one or more culture conditions that may alter the protein profile of the cells for a given period of time. The cells are collected and washed with ice-cold H/H that includes 10 mM sodium pyrophosphate, 10 mM sodium fluoride, 10 mM EDTA, and 1 mM sodium orthovanadate. The cells are then lysed in lysis buffer (50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Triton X-100, 10 mM sodium pyrophosphate, 10mM sodium fluoride, 10 mM EDTA, 1 mM sodium orthovanadate, 1  $\mu$ g/mL PMSF, 1  $\mu$ g/mL aprotinin, 1  $\mu$ g/mL leupeptin, and 1  $\mu$ g/mL pepstatin A) by gentle mixing, and placed on ice for 5 minutes. After lysis, the lysate is transferred to centrifuge tubes and centrifuged in an ultracentrifuge at 75000 rpm for 15 min at 4°C. The supernatant is transferred to eppendorf tubes and pre-cleared with 10  $\mu$ l of rabbit pre-immune antibody on a rotator at 4°C for 1 hr. Forty  $\mu$ l of protein A-Sepharose (Amersham) is then added and incubated at 4°C overnight on a rotator.

The protein A-Sepharose beads are harvested and the supernatant removed to a fresh eppendorf tube. Immune antibody is added to supernatant and rotated for 1 hr at 4°C. Thirty  $\mu$ l of protein A-Sepharose is then added and the mixture is further rotated at 4°C for 1 hr. The beads are harvested and the supernatant is aspirated. The beads are washed three times with 50 mM Tris (pH 8.0), 150 mM NaCl, 0.1% Triton X-100, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 10 mM sodium orthovanadate, and 10 mM EDTA. Dry the beads with a 50  $\mu$ l Hamilton syringe. Laemmli loading buffer containing 100 mM

DTT is added to the beads and samples are boiled for 5 min. The beads are spun down and the supernatant is loaded onto SDS-PAGE gels. Comparison of the control and experimental samples allows for the selection of polypeptides that interact with the protein of interest.

5

*EXAMPLE 13 Sample for Mass Spectrometry of Interacting Proteins*

The gel slices are cut into 1 mm cubes and 10 to 20  $\mu$ l of 1% acetic acid is added. The gel particles are washed with 100 - 150  $\mu$ l of HPLC grade water (5 minutes with occasional mixing), briefly centrifuged, and the liquid is removed. Acetonitrile (~200  $\mu$ l, 10 approximately 3 to 4 times the volume of the gel particles) is added followed by incubation at room temperature for 10 to 15 minutes with vortexing. A second acetonitrile wash may be required to completely dehydrate the gel particles. The sample is briefly centrifuged and all the liquid is removed.

The protein in the gel particles is reduced at 50 degrees Celsius using 10 mM 15 dithiothreitol (in 100 mM ammonium bicarbonate) for 30 minutes and then alkylated at room temperature in the dark using 55 mM iodoacetamide (in 100 mM ammonium bicarbonate). The gel particles are rinsed with a minimal volume of 100 mM ammonium bicarbonate before a trypsin (50 mM ammonium bicarbonate, 5 mM CaCl<sub>2</sub>, and 12.5 ng/ $\mu$ l trypsin) solution is added. The gel particles are left on ice for 30 to 45 minutes (after 20 20 minutes incubation more trypsin solution is added). The excess trypsin solution is removed and 10 to 15  $\mu$ l digestion buffer without trypsin is added to ensure the gel particles remain hydrated during digestion. The samples are digested overnight at 37°C.

The following day, the supernatant is removed from the gel particles. The peptides are extracted from the gel particles with 2 changes of 100  $\mu$ L of 100 mM ammonium 25 bicarbonate with shaking for 45 minutes and pooled with the initial gel supernatant. The extracts are acidified to 1% (v/v) with 100% acetic acid.

(a) Method One for Purification of Tryptic Peptides

The tryptic peptides are purified with a C18 reverse phase resin. 250  $\mu$ L of dry resin is washed twice with methanol and twice with 75% acetonitrile/1% acetic acid. A 5:1 30 slurry of solvent : resin is prepared with 75% acetonitrile/1% acetic acid. To the extracted peptides, 2  $\mu$ L of the resin slurry is added and the solution is shaken at moderate speed for 30 minutes at room temperature. The supernatant is removed and replaced with 200  $\mu$ L of

2% acetonitrile/1% acetic acid and shaken for 5-15 minutes with moderate speed. The supernatant is removed and the peptides are eluted from the resin with 15 µL of 75% acetonitrile/1% acetic acid with shaking for about 5 minutes. The peptide and slurry mixture is applied to a filter plate and centrifuged for 1-2 minutes at 1000 rpm, the filtrate is  
5 collected and stored at -70°C until use.

(b) Method Two for Purification of Tryptic Peptides

Alternatively, the tryptic peptides may be purified using ZipTip<sub>c18</sub> (Millipore, Cat # ZTC18S960). The ZipTips are first pre-wetted by aspirating and dispensing 100% methanol 5 times. The tips are then washed with 2% acetonitrile/1% acetic acid (5 times),  
10 followed by 65% acetonitrile/1% acetic (5 times) and returned to 2% acetonitrile/1% acetic acid (5 times). The ZipTips are replaced in their rack and the residual solvent is eliminated. The ZipTips are washed again with 2% acetonitrile/1% acetic acid (5 times). The digested peptides are bound to the ZipTips by aspirating and dispensing the samples 5 times. Salts are removed by washing ZipTips with 2% acetonitrile/1% acetic acid (5 times). 10 µL of  
15 65% acetonitrile/1% acetic acid is collected by the ZipTips and dispensed into a 96-well microtitre plate. 1 µL of sample and 1 µL of matrix are spotted on a MALDI-TOF sample plate for analysis.

*EXAMPLE 14 Mass Spectrometric Analysis of Interacting Proteins*

(a) Method One for Analysis of Tryptic Peptides

Analytical samples containing tryptic peptides are subjected to Matrix Assisted Laser Desorption/Ionization Time Of Flight (MALDI-TOF) mass spectrometry. Samples are mixed 1:1 with a matrix of α-cyano-4-hydroxy-*trans*-cinnamic acid. The sample/matrix mixture is spotted on to the MALDI sample plate with a robot. The sample/matrix mixture  
25 is allowed to dry on the plate and is then introduced into the mass spectrometer. Analysis of the peptides in the mass spectrometer is conducted using both delayed extraction mode and an ion reflector to ensure high resolution of the peptides.

Internally-calibrated tryptic peptide masses are searched against both in-house proprietary and public databases using a correlative mass matching algorithm. Statistical  
30 analysis is performed on each protein match to determine its validity. Typical search constraints include error tolerances within 0.1 Da for monoisotopic peptide masses and

carboxyamidomethylation of cysteines. Identified proteins are stored automatically in a relational database with software links to SDS-PAGE images and ligand sequences.

(b) Method Two for Analysis of Tryptic Peptides

Alternatively, samples containing tryptic peptides are analyzed with an ion trap instrument. The peptide extracts are first dried down to approximately 1  $\mu$ L of liquid. To this, 0.1% trifluoroacetic acid (TFA) is added to make a total volume of approximately 5  $\mu$ L. Approximately 1-2  $\mu$ L of sample are injected onto a capillary column (C8, 150  $\mu$ m ID, 15 cm long) and run at a flow rate of 800 nL/min. using the following gradient program:

Time (minutes)	% Solvent A	% Solvent B
0	95	5
30	65	35
40	20	80
41	95	5

Where Solvent A is composed of water/0.5% acetic acid and Solvent B is acetonitrile/0.5% acetic acid. The majority of the peptides will elute between the 20-40 % acetonitrile gradient. Two types of data from the eluting HPLC peaks are acquired with the ion trap mass spectrometer. In the MS<sup>1</sup> dimension, the mass to charge range for scanning is set at 400-1400 - this will determine the parent ion spectrum. Secondly, the instrument has MS<sup>2</sup> capabilities whereby it will acquire fragmentation spectra of any parent ions whose intensities are detected to be greater than a predetermined threshold (Mann and Wilm, *Anal Chem* 66(24): 4390-4399 (1994)). A significant amount of information is collected for each protein sample as both a parent ion spectrum and many daughter ion spectra are generated with this instrumentation.

All resulting mass spectra are submitted to a database search algorithm for protein identification. A correlative mass algorithm is utilized along with a statistical verification of each match to identify a protein's identification (Ducret A, et al., *Protein Sci* 7(3): 706-719 (1998)). This method proves much more robust than MALDI-TOF mass spectrometry for identifying the components of complex mixtures of proteins.

No interacting proteins were observed using at least one of the methods described above.

*EXAMPLE 15 NMR Analysis*

Purified protein sample is centrifuged at 13,000 rpm for 10 minutes with a bench-top microcentrifuge to eliminate any precipitated protein. The supernatant is then transferred into a clean tube and the sample volume is measured. If the sample volume is 5 less than 450  $\mu$ l, an appropriate amount of crystal buffer is added to the sample to reach that volume. Then 50  $\mu$ l of D<sub>2</sub>O (99.9%) is added to the sample to make an NMR sample of 500  $\mu$ l. The usual concentration of the protein sample is usually approximately 1 mmol or greater.

NMR screening experiments are performed on a Bruker AV600 spectrometer 10 equipped with a cryoprobe, or other equivalent instrumentation. All spectra are recorded at 25°C. Standard 1D proton pulse sequence with presaturation is used for 1D screening. Normally, a sweepwidth of 6400 Hz, and eight or sixteen scans are used, although different pulse sequences are known to those of skill in the art and may be readily determined. For <sup>1</sup>H, <sup>15</sup>N HSQC experiments, a pulse sequence with "flip-back" water suppression may be 15 used. Typically, sweepwidths of 8000 Hz and 2000 Hz are used for F2 and F1 dimension, respectively. Four to sixteen scans are normally adequate. The data is then processed on a Sun Ultra 5 computer with NMRpipe software.

*EXAMPLE 16 X-ray Crystallography*

## 20 (a) Crystallization

Subsequent to purification, a subject polypeptide is centrifuged for 10 minutes at 4°C and at 14,000 rpm in order to sediment any aggregated protein. The protein sample is then diluted in order to provide multiple concentrations for screening.

Two 96 well plates (Nunc) are employed for the initial crystal screen, with 48 25 potential crystallization conditions. The screening library has crystallization conditions found in Hampton Research Crystal Screen I (Jankarik, J. and S.H. Kim, J. Appl. Cryst., 1991. 24:409-11), Hampton Research Crystal Screen II, Hampton Crystal Screen I-Lite, and from Emerald Biostructures, Inc., Bainbridge Island, WA, Wizard I, Wizard II, Cryo I and Cryo II. Alternatively, other conditions known to those of skill in the art, including 30 those provided in screening kits available from other companies, may also be tested.

Conditions are tested at multiple protein concentrations and at two temperatures (4 and 20°C). Crystal setups may be performed by a liquid handling robot appropriately

programmed for sitting drop experiments. The robot loads 50  $\mu$ l of buffer into each screening well on a 24 or 96 well sitting drop crystal screen tray, and then loads 1 - 5  $\mu$ l of protein into each drop reservoir to be screened on the plate. Subsequently, the robot loads 1.5  $\mu$ l of the corresponding screening solution into the drop reservoir atop the protein. The 5 plate is then sealed using transparent tape, and stored at 4 or 20°C. Each plate is observed two days, two weeks, and 1 month after being set. Alternatively, screens may be performed using 0.1 - 10  $\mu$ l drops suspended at the interface of two immiscible oils. The protein containing solution has a density intermediate between the two oils and thus floats between them (Chayen N.E.: 1996, *Protein Eng.* 9:927-29). This procedure may be performed in an 10 automated fashion by an appropriately programmed liquid handling robot, with additional steps being required initially to introduce the oils. No tape is added to facilitate gradual drying out of the drop to promote crystallization.

Having identified conditions that are best suited for further crystal refinement, subsequent plates are set up to explore the affects of variables such as temperature, pH, salt 15 or PEG concentration on crystal size and form, with the intent of establishing conditions where the protein is able to form crystals of suitable size and morphology for diffraction analysis. Each refinement is performed in the sitting drop format in a 24 well Lindbro plate. Each well in the tray contains 500  $\mu$ l of screening solution, and a 1.5  $\mu$ l drop of protein diluted with 1.5  $\mu$ l of the screening solution is set to hang from the siliconized glass 20 cover slip covering the well. Alternatively, refinement steps may be performed using either the machine 96 well plate hanging drop method or the oil suspension method described above.

Suitable crystals for x-ray experimentation were obtained by using 11 mg/ml protein 20% PEG4000 as precipitant, 6.4% isopropanol, 2% MPD and 100mM HEPES, pH 7.4. 25 The crystals are somewhat sensitive to changes in their solution and care is required to freeze them without the crystals cracking. The crystal was stabilized in a mixture of 20% (v/v) ethylene glycol in the mother liquor. A slow soak protocol was used wherein ethylene glycol and the mother liquor were mixed in a 1:3 volume ratio, and 5 microlitres were added on top of the skin that covers the surface of the drop containing the crystals. This 30 skin was then punctured at a location remote from the crystal, and the two liquids allowed to mix. After a few minutes of passive mixing, a loop was used to ensure that the liquids

were thoroughly mixed. The crystals were frozen at 100K in a cold gas stream generated by an X-stream 2000 cryocooling device.

(b) Co-Crystallization

A variety of methods known in the art may be used for preparation of co-crystals comprising the subject polypeptides and one or more compounds that interact with the subject polypeptides, such as, for example, an inhibitor, co-factor, substrate, polynucleotide, polypeptide, and/or other molecule. In one exemplary method, crystals of the subject polypeptide may be soaked, for an appropriate period of time, in a solution containing a compound that interacts with a subject polypeptide. In another method, solutions of the subject polypeptide and/or compound that interacts with the subject polypeptide may be prepared for crystallization as described above and mixed into the above-described sitting drops. In certain embodiments, the molecule to be co-crystallized with the subject polypeptide may be present in the buffer in the sitting drop prior to addition of the solution comprising the subject polypeptide. In other embodiments, the subject polypeptide may be mixed with another molecule before adding the mixture to the sitting drop. Based on the teachings herein, one of skill in the art may determine the co-crystallization method yielding a co-crystal comprising the subject polypeptide.

(c) Heavy Atom Substitution

For preparation of crystals containing heavy atoms, crystals of the subject polypeptide may be soaked in a solution of a compound containing the appropriate heavy atom for such period as time as may be experimentally determined is necessary to obtain a useful heavy atom derivative for x-ray purposes. Likewise, for other compounds that may be of interest, including, for example, inhibitors or other molecules that interact with the subject polypeptide, crystals of the subject polypeptide may be soaked in a solution of such compound for an appropriate period of time.

(d) Data collection and processing

Native data were collected from a single frozen crystal using a monochromatic CuK x-rays (Bruker X-ray generator) and a SMART 6000 CCD detector. The crystal diffracted to a minimum d-spacing of 1.9 Å. All data were processed using the SAINT/PROSCALE software package. The protein atom coordinates of the *E. coli* PTH (Protein data bank code 2PTH) were used as the search model for molecular replacement using CNX. This yielded a solution with a correlation coefficient of 50.5 and a packing coefficient of 67.9. The structure was refined using the CNX program suite. Ten percent

of the reflections were randomly excluded from the refinement, and used to monitor Rfree. A maximum likelihood target (with a flat bulk solvent correction and no low resolution or sigma cutoff applied to the data) was used in the refinement protocol. Refinement of the model using the simulated annealing slow-cooling and individual temperature factor refinement protocol was alternated with manual inspection and rebuilding of the model using TURBO-FRODO (Roussel A. et Cambillau C. (1989) TURBO-FRODO. Silicon Graphics Geometry Partner Directory, Silicon Graphics, Mountain view, California). After several cycles of refinement and manual rebuilding, 192 residues of the protein monomer were modeled, with residues 193 – 194 being disordered. In addition to the protein, the structure also contains 215 solvent molecules and one ethylene glycol molecule.

Structure solution and refined statistics for are reported in Table 3, contained in FIGURE 8. FIGURE 9 contains a list of the atomic coordinates of the subject polypeptide and other molecules contained in the crystal. FIGURE 10 to FIGURE 14 depict various features of the crystal structure and other properties of a subject polypeptide.

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(e) Analysis of the X-ray Structure of the Subject Polypeptide

*General Description of Structure*

Although the crystal used contains two molecules in the asymmetric unit, PTH behaves in solution as a monomer of 193 residues. The R.M.S deviation between the two monomers of the asymmetric unit is 0.23 Å for 193 C atoms showing that the two monomers are essentially identical. The PA enzyme structure is composed of a single / globular domain build around a twisted -sheet (FIGURE 11).

*Active Site and Other Druggable Regions*

The amino acid sequences for the druggable regions that are two active binding pockets of the enzyme revealed by the *P. aeruginosa* structure using the Site Finder functionality in a software called MOE (Chemical Computing Group) are indicated below:

<u>Binding Site Identifier</u>	<u>Amino Acid Residues</u>	<u>Description of Binding Site</u>
Binding Site I	N12, Y17, H22, Y68, M69, N70, D95, L97, G114, H115, N116, G117, V147, S148, V151, L152	Peptide binding/catalytic site. This pocket is approximately 105 units (Å <sup>3</sup> ).
Binding Site II	A11, N12, P13, G14, P15, Y17, D18, N19,	tRNA binding site. This pocket is approximately 171

	T20, R21, N23, A24, A26, R27, L49, I64, P65, T66, G153, R154, A155, Q160, L163	units ( $\text{\AA}^3$ ).
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In the *E. coli* enzyme, the peptide binding site pocket extends for 255 units, compared to 105 units ( $\text{\AA}^3$ ) in the *P. aeruginosa*. Furthermore, the tRNA binding site in *E. coli* appears to be split into two (85 and 87 units) due to the presence of a tryptophan residue in the narrow region of the channel (FIGURE 14). The specific presence of a bulky residue in the *E. coli* PTH (FIGURE 14) partially occluding the mid-section of the active site channel suggests that conformational changes may be required upon tRNA-PTH complex formation to accommodate tRNAs carrying longer peptides.

The putative catalytic sites of the *P. aeruginosa* enzyme located on both edges of the protein dimer are highly conserved to the ones found in the *E. coli* monomeric protein (FIGURE 13).

#### *Comparison to Other Peptidyl-tRNA Hydrolase Enzymes*

The superimposition of the *P. aeruginosa* and *E. coli* PTHs produces a final r.m.s. deviation of 1.1  $\text{\AA}$  for 188 pairs of C compared (FIGURE 12). Residues shown to be involved in catalysis or tRNA substrate binding are extremely well conserved among the five bacteria (alignment shown in FIGURE 10 with the exception of K142, shown to be involved in the tRNA binding in the *E. coli* PTH that is part of a region making a flexible lid over the active site crevice. The conservation as determined from this alignment for PTH residues involved in catalysis or tRNA binding are shown below:

20

Conservation of PTH residues shown to be involved in catalysis or tRNA binding					
Residues	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>H. pylori</i>	<i>S. aureus</i>	<i>S. pneumonia</i>
Asn12	Conserved	Conserved	Conserved	Conserved	Conserved
His22	Conserved	Conserved	Conserved	Conserved	Conserved
Met69	Conserved	Conserved	Conserved	Conserved	Conserved
Asn70	Conserved	Conserved	Conserved	Conserved	Conserved
Asp95	Conserved	Conserved	Conserved	Conserved	Conserved
His115	Conserved	Conserved	Conserved	Conserved	Conserved

Asn116	Conserved	Conserved	Conserved	Conserved	Conserved
Arg135	Conserved	Conserved	Conserved	Conserved	Conserved
Lys144	Ser	Lys	Deletion	Gly	Gly

Based in part on the structural information described above, in one aspect, the present invention is directed to a druggable region of a polypeptide of the invention containing a druggable region for binding a polypeptide with a volume of less than 5 approximately 200, 150 or 125 units ( $\text{\AA}^3$ ). In one embodiment, the druggable region comprises at least one of residues Asn12, Tyr17, His22, Tyr68, Met69, Asn70, Asp95, Leu97, Gly114, His115, Asn116, Gly117, Val147, Ser148, Val151, or Leu152. In another embodiment, the druggable region comprises at least one of residues Ala11, Asn12, Pro13, Gly14, Pro15, Tyr17, Asp18, Asn19, Thr20, Arg21, Asn23, Ala24, Ala26, Arg27, Leu49, 10 Ile64, Pro65, Thr66, Gly153, Arg154, Ala155, Gln160, or Leu163. In still another embodiment, the druggable region may comprise at least one of residues Asn12, His22, Met69, Asn70, Asp95, His115, Asn116, Arg135, or Lys144. In another aspect, the present invention is directed to a druggable region of a peptidyl-tRNA hydrolase for binding tRNA that (i) does not contain any amino acid residue, such as tryptophan or an amino acid 15 residue containing an aromatic group or a large aliphatic nonpolar group, or (ii) that consists of a single pocket with a volume of at least 100 or 150 units ( $\text{\AA}^3$ ). In another aspect, the present invention is directed to a peptidyl-tRNA hydrolase that does not require a conformational change to form a complex with tRNA. In another aspect, the present invention is directed to a peptidyl-tRNA hydrolase that is a dimer of two monomers in its 20 naturally occurring active form.. In another aspect, the present invention is directed toward an inhibitor that interacts with the active site of such an enzyme. In one embodiment, an inhibitor may modulate the conformation change in a peptidyl-tRNA hydrolase.

#### *EXAMPLE 17 Annotations*

25 The functional annotation is arrived at by comparing the amino acid sequence of the ORF against all available ORFs in the NCBI database using BLAST. The closest match is selected to provide the probable function of the polypeptide having the sequence of SEQ ID NO: 2. Results of this comparison are described above and set forth in Table 2 of FIGURE 7.

The COGs database (Tatusov RL, Koonin EV, Lipman DJ. *Science* 1997; 278 (5338) 631-37) classifies proteins encoded in twenty-one completed genomes on the basis of sequence similarity. Members of the same Cluster of Orthologous Group, ("COG"), are expected to have the same or similar domain architecture and the same or substantially similar biological activity. The database may be used to predict the function of uncharacterised proteins through their homology to characterized proteins. The COGs database may be searched from NCBI's website (<http://www.ncbi.nlm.nih.gov/COG/>) to determine functional annotation descriptions, such as "information storage and processing" (translation, ribosomal structure and biogenesis, transcription, DNA replication, recombination and repair); "cellular processes" (cell division and chromosome partitioning, post-translational modification, protein turnover, chaperones, cell envelope biogenesis, outer membrane, cell motility and secretion, inorganic ion transport and metabolism, signal transduction mechanisms); or "metabolism" (energy production and conversion, carbohydrate transport and metabolism, amino acid transport and metabolism, nucleotide transport and metabolism, coenzyme metabolism, lipid metabolism). For certain polypeptides, there is no entry available. Results of this analysis are described above and set forth in Table 2 of FIGURE 7.

*EXAMPLE 18 Essential Gene Analysis*

SEQ ID NO: 2 is compared to a number of publicly available "essential genes" lists to determine whether that protein is encoded by an essential gene. An example of such a list is descended from a free release at the [www.shigen.nig.ac.jp](http://www.shigen.nig.ac.jp) PEC (profiling of *E. coli* chromosome) site, <http://www.shigen.nig.ac.jp/ecoli/pec/>. The list is prepared as follows: a wildcard search for all genes in class "essential" yields the list of essential *E. coli* proteins encoded by essential genes, which number 230. These 230 hits are pruned by comparing against an NCBI *E. coli* genome. Only 216 of the 230 genes on the list are found in the NCBI genome. These 216 are termed the essential-216-*ecoli* list. The essential-216-*ecoli* list is used to garner "essential" genes lists for other microbial genomes by blasting. For instance, formatting the 216-*ecoli* as a BLAST database, then BLASTing a genome (e.g. *S. aureus*) against it, elucidates all *S. aureus* genes with significant homology to a gene in the 216-essential list. SEQ ID NO: 2 is compared against the appropriate list and a match with a score of  $e^{-25}$  or better is considered an essential gene according to that list. In addition to the list described above, other lists of essential genes are publicly available or may be

determined by methods disclosed publicly, and such lists and methods are considered in deciding whether a gene is essential. See, for example, Thanassi et al., Nucleic Acids Res 2002 Jul 15;30(14):3152-62; Forsyth et al., Mol Microbiol 2002 Mar;43(6):1387-400; Ji et al., Science 2001 Sep 21;293(5538):2266-9; Sassetti et al., Proc Natl Acad Sci U S A 2001 5 Oct 23;98(22):12712-7; Reich et al., J Bacteriol 1999 Aug;181(16):4961-8; Akerley et al., Proc Natl Acad Sci U S A 2002 Jan 22;99(2):966-71). Also, other methods are known in the art for determining whether a gene is essential, such as that disclosed in U.S. Patent Application No. 10/202,442 (filed July 24, 2002). The conclusion as to whether the gene encoding the amino acid sequence set forth in SEQ ID NO: 2 is essential is set forth in 10 Table 2 of FIGURE 7.

#### *EXAMPLE 19 PDB Analysis*

SEQ ID NO: 2 is compared against the amino acid sequences in a database of proteins whose structures have been solved and released to the PDB (protein data bank). 15 The identity/information about the top PDB homolog (most similar "hit", if any; a PDB entry is only considered a hit if the score is  $e^4$  or better) is annotated, and the percent similarity and identity between SEQ ID NO: 2 and the closest hit is calculated, with both being indicated in Table 2 of FIGURE 7.

#### *EXAMPLE 20 Virtual Genome Analysis*

VGDB or VG is a queryable collection of microbial genome databases annotated with biophysical and protein information. The organisms present in VG include:

File	GRAM	Species	Source	Genome file date
ecoli.faa	G-	<i>Escherichia coli</i>	NCBI	November 18 1998
hpyl.faa	G-	<i>Helicobacter pylori</i>	NCBI	April 19 1999
		<i>Pseudomonas</i>		
paer.faa	G-	<i>aeruginosa</i>	NCBI	September 22 2000
ctra.faa	G-	<i>Chlamydia trachomatis</i>	NCBI	December 22 1999
hinf.faa	G-	<i>Haemophilus influenzae</i>	NCBI	November 26 1999
nmen.faa	G-	<i>Neisseria meningitidis</i>	NCBI	December 28 2000
rpxx.faa	G-	<i>Rickettsia prowazekii</i>	NCBI	December 22 1999
bbur.faa	G-	<i>Borrelia burgdorferi</i>	NCBI	November 11 1998
bsub.faa	G+	<i>Bacillus subtilis</i>	NCBI	December 1 1999
staph.faa	G+	<i>Staphylococcus aureus</i>	TIGR	March 8 2001
		<i>Streptococcus</i>		
spne.faa	G+	<i>pneumoniae</i>	TIGR	February 22 2001
mgen.faa	G+	<i>Mycoplasma genitalium</i>	NCBI	November 23 1999

efae.faa G+

*Enterococcus faecalis* TIGR

March 8 2001

The VGDB comprises 13 microbial genomes, annotated with biophysical information (pI, MW, etc), and a wealth of other information. These 13 organism genomes are stored in a single flatfile (the VGDB) against which PSI-blast queries can be done.

5 SEQ ID NO: 2 is queried against the VGDB to determine whether this sequence is found, conserved, in many microbial genomes. There are certain criteria that must be met for a positive hit to be returned (beyond the criteria inherent in a basic PSI-blast).

When an ORF is queried it may have a maximum of 13 VG-organism hits. A hit is classified as such as long as it matches the following criteria: Minimum Length (as 10 percentage of query length): 75 (*Ensure hit protein is at least 75% as long as query*); Maximum Length (as percentage of query length): 125 (*Ensure hit protein is no more than 125% as long as query*); eVal:-10 (*Ensure hit has an e-Value of e-10 or better*); Id%:>.25 (*Ensure hit protein has at least 25% identity to query*). The e-Value is a standard parameter of BLAST sequence comparisons, and represents a measure of the similarity between two 15 sequences based on the likelihood that any similarities between the two sequences could have occurred by random chance alone. The lower the e-Value, the less likely that the similarities could have occurred randomly and, generally, the more similar the two sequences are.

The organisms having an orthologue of the polypeptide having SEQ ID NO: 2 are 20 listed in Table 2, shown in FIGURE 7.

#### ***EXAMPLE 21 Epitopic Regions***

The three most likely epitopic regions of a polypeptide having SEQ ID NO: 2 are predicted using the semi-empirical method of Kolaskar and Tongaonkar (FEBS Letters 25 1990 v276 172-174), the software package called Protean (DNASTAR), or MacVectors's Protein analysis tools (Accerlyrs). The antigenic propensity of each amino acid is calculated by the ratio between frequency of occurrence of amino acids in 169 antigenic determinants experimentally determined and the calculated frequency of occurrence of amino acids at the surface of protein. The results of these bioinformatics analyses are 30 presented in Table 2, shown in FIGURE 7.

## EQUIVALENTS

The present invention provides among other things, novel proteins, protein structures and protein-protein interactions. While specific embodiments of the subject invention have been discussed, the above specification is illustrative and not restrictive.

5 Many variations of the invention will become apparent to those skilled in the art upon review of this specification. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

All publications and patents mentioned herein, including those items listed below,  
10 are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control. To the extent that any U.S. Provisional Patent Applications to which this patent application claims priority incorporate by reference another U.S. Provisional Patent Application, such other  
15 U.S. Provisional Patent Application is not incorporated by reference herein unless this patent application expressly incorporates by reference, or claims priority to, such other U.S. Provisional Patent Application.

Also incorporated by reference in their entirety are any polynucleotide and polypeptide sequences which reference an accession number correlating to an entry in a public database, such as those maintained by The Institute for Genomic Research (TIGR) ([www.tigr.org](http://www.tigr.org)) and/or the National Center for Biotechnology Information (NCBI) ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)).

Also incorporated by reference are the following: WO 00/45168, WO 00/79238, WO 00/77712, EP 1047108, EP 1047107, WO 00/72004, WO 00/73787, WO 00/67017,  
25 WO 00/48004, WO 01/48209, WO 00/45168, WO 00/45164, U.S.S.N. 09/720272; PCT/CA99/00640; U.S. Patent Application Nos: 10/097125 (filed March 12, 2002); 10/097193 (filed March 12, 2002); 10/202442 (filed July 24, 2002); 10/097194 (filed March 12, 2002); 09/671817 (filed September 17, 2000); 09/965654 (filed September 27, 2001); 09/727812 (filed November 30, 2000); 60/370667 (filed April 8, 2002); a utility  
30 patent application entitled "Methods and Apparatuses for Purification" (filed September 18, 2002); U.S. Patent Numbers 6451591; 6254833; 6232114; 6229603; 6221612; 6214563; 6200762; 6171780; 6143492; 6124128; 6107477; D428157; 6063338; 6004808; 5985214; 5981200; 5928888; 5910287; 6248550; 6232114; 6229603; 6221612; 6214563; 6200762;

6197928; 6180411; 6171780; 6150176; 6140132; 6124128; 6107066; 6270988; 6077707;  
6066476; 6063338; 6054321; 6054271; 6046925; 6031094; 6008378; 5998204; 5981200;  
5955604; 5955453; 5948906; 5932474; 5925558; 5912137; 5910287; 5866548; 6214602;  
5834436; 5777079; 5741657; 5693521; 5661035; 5625048; 5602258; 5552555; 5439797;  
5 5374710; 5296703; 5283433; 5141627; 5134232; 5049673; 4806604; 4689432; 4603209;  
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We claim:

## CLAIMS

1. A composition comprising an isolated, recombinant polypeptide, wherein the polypeptide comprises: (a) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (b) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (c) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*; and 10 wherein the polypeptide of (a), (b) or (c) is at least about 90% pure in a sample of the composition.
2. The composition of claim 1, wherein the polypeptide is at least about 95% pure as determined by gel electrophoresis.
3. The composition of claim 1, wherein the polypeptide is purified to essential 15 homogeneity.
4. The composition of claim 1, wherein at least about two-thirds of the polypeptide in the sample is soluble.
5. The composition of claim 1, wherein the polypeptide is fused to at least one heterologous polypeptide that increases the solubility or stability of the polypeptide.
- 20 6. The composition of claim 1, which further comprises a matrix suitable for mass spectrometry.
7. The composition of claim 6, wherein the matrix is a nicotinic acid derivative or a cinnamic acid derivative.
8. A sample comprising an isolated, recombinant polypeptide, wherein the 25 polypeptide comprises: (a) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (b) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (c) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has 30 at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*; and wherein the polypeptide of (a), (b) or (c) is labeled with a heavy atom.
9. The sample of claim 8, wherein the heavy atom is one of the following: cobalt, selenium, krypton, bromine, strontium, molybdenum, ruthenium, rhodium, palladium,

silver, cadmium, tin, iodine, xenon, barium, lanthanum, cerium, praseodymium, neodymium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, lutetium, tantalum, tungsten, rhenium, osmium, iridium, platinum, gold, mercury, thallium, lead, thorium and uranium.

5        10. The sample of claim 8, wherein the polypeptide is labeled with seleno-methionine.

11. The sample of claim 8, further comprising a cryo-protectant.

12. The sample of claim 11, wherein the cryo-protectant is one of the following:  
methyl pentanediol, isopropanol, ethylene glycol, glycerol, formate, citrate, mineral oil and  
10      a low-molecular-weight polyethylene glycol.

13. A crystallized, recombinant polypeptide comprising: (a) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (b) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (c) an amino acid sequence encoded by a polynucleotide that hybridizes under  
15      stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*; wherein the polypeptide of (a), (b) or (c) is in crystal form.

14. A crystallized complex comprising the crystallized, recombinant polypeptide of claim 13 and a co-factor, wherein the complex is in crystal form.

20        15. A crystallized complex comprising the crystallized, recombinant polypeptide of claim 13 and a small organic molecule, wherein the complex is in crystal form.

16. The crystallized, recombinant polypeptide of claim 13, which diffracts x-rays to a resolution of about 3.5 Å or better.

25        17. The crystallized, recombinant polypeptide of claim 13, wherein the polypeptide comprises at least one heavy atom label.

18. The crystallized, recombinant polypeptide of claim 17, wherein the polypeptide is labeled with seleno-methionine.

19. A method for designing a modulator for the prevention or treatment of *P. aeruginosa* related disease or disorder, comprising:

30        (a) providing a three-dimensional structure for a crystallized, recombinant polypeptide of claim 13;

(b) identifying a potential modulator for the prevention or treatment of *P. aeruginosa* related disease or disorder by reference to the three-dimensional structure;

(c) contacting a polypeptide of the composition of claim 1 or *P. aeruginosa* with the potential modulator; and

5 (d) assaying the activity of the polypeptide or determining the viability of *P. aeruginosa* after contact with the modulator, wherein a change in the activity of the polypeptide or the viability of *P. aeruginosa* indicates that the modulator may be useful for prevention or treatment of a *P. aeruginosa* related disease or disorder.

10 20. A sample comprising an isolated, recombinant polypeptide, wherein the polypeptide comprises: (a) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (b) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (c) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has 15 at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*; and wherein the polypeptide of (a), (b) or (c) is enriched in at least one NMR isotope.

21. The sample of claim 20, wherein the NMR isotope is one of the following: hydrogen-1 (<sup>1</sup>H), hydrogen-2 (<sup>2</sup>H), hydrogen-3 (<sup>3</sup>H), phosphorous-31 (<sup>31</sup>P), sodium-23 (<sup>23</sup>Na), nitrogen-14 (<sup>14</sup>N), nitrogen-15 (<sup>15</sup>N), carbon-13 (<sup>13</sup>C) and fluorine-19 (<sup>19</sup>F).

20 22. The sample of claim 20, further comprising a deuterium lock solvent.

23. The sample of claim 22, wherein the deuterium lock solvent is one of the following: acetone (CD<sub>3</sub>COCD<sub>3</sub>), chloroform (CDCl<sub>3</sub>), dichloro methane (CD<sub>2</sub>Cl<sub>2</sub>), methylnitrile (CD<sub>3</sub>CN), benzene (C<sub>6</sub>D<sub>6</sub>), water (D<sub>2</sub>O), diethylether ((CD<sub>3</sub>CD<sub>2</sub>)<sub>2</sub>O), dimethylether ((CD<sub>3</sub>)<sub>2</sub>O), N,N-dimethylformamide ((CD<sub>3</sub>)<sub>2</sub>NCDO), dimethyl sulfoxide 25 (CD<sub>3</sub>SOCD<sub>3</sub>), ethanol (CD<sub>3</sub>CD<sub>2</sub>OD), methanol (CD<sub>3</sub>OD), tetrahydrofuran (C<sub>4</sub>D<sub>8</sub>O), toluene (C<sub>6</sub>D<sub>5</sub>CD<sub>3</sub>), pyridine (C<sub>5</sub>D<sub>5</sub>N) and cyclohexane (C<sub>6</sub>H<sub>12</sub>).

24. The sample of claim 20, which is contained within an NMR tube.

25. A method for identifying small molecules that bind to a polypeptide of the composition of claim 1, comprising:

30 (a) generating a first NMR spectrum of an isotopically labeled polypeptide of the composition of claim 1;

(b) exposing the polypeptide to one or more small molecules;

(c) generating a second NMR spectrum of the polypeptide which has been exposed to one or more small molecules; and

(d) comparing the first and second spectra to determine differences between the first and the second spectra, wherein the differences are indicative of one or more small  
5 molecules that have bound to the polypeptide.

26. A host cell comprising a nucleic acid encoding a polypeptide comprising: (a) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (b) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (c) an amino acid sequence encoded by a polynucleotide  
10 that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*; wherein a culture of the host cell produces at least about 1 mg of the polypeptide per liter of culture and the polypeptide is at least about one-third soluble as measured by gel electrophoresis.

15 27. An isolated, recombinant polypeptide, comprising: (a) an amino acid sequence having at least about 90% identity with the amino acid sequence set forth in SEQ ID NO: 4; or (b) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P.  
20 aeruginosa*; and wherein the polypeptide comprises one or more of the following amino acid residues at the specified position of the polypeptide: Asn12, His22, Met69, Asn70, Asp95, His115, Asn116, and Arg135.

28. A method for obtaining structural information of a crystallized polypeptide, the method comprising:

25 (a) crystallizing a recombinant polypeptide, wherein the polypeptide comprises: (1) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (2) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (3) an amino acid sequence encoded by a polynucleotide  
30 that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*; and wherein the crystallized polypeptide is capable of diffracting X-rays to a resolution of 3.5 Å or better; and

(b) analyzing the crystallized polypeptide by X-ray diffraction to determine the three-dimensional structure of at least a portion of the crystallized polypeptide.

29. The method of claim 28, wherein the three-dimensional structure of the portion of the crystallized polypeptide is determined to a resolution of 3.5 Å or better.

5 30. A method for identifying a druggable region of a polypeptide, the method comprising:

(a) obtaining crystals of a polypeptide comprising (1) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (2) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or  
10 (3) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*, such that the three dimensional structure of the crystallized polypeptide may be determined to a resolution of 3.5 Å or better;

15 (b) determining the three dimensional structure of the crystallized polypeptide using X-ray diffraction; and

(c) identifying a druggable region of the crystallized polypeptide based on the three-dimensional structure of the crystallized polypeptide.

31. The method of claim 30, wherein the druggable region is an active site.

20 32. The method of claim 31, wherein the druggable region is on the surface of the polypeptide.

33. Crystalline peptidyl-tRNA hydrolase from *P. aeruginosa* comprising a crystal having unit cell dimensions  $a = 63.702 \text{ \AA}$ ,  $b = 63.702 \text{ \AA}$ ,  $c = 154.844 \text{ \AA}$ ,  $\alpha = \beta = 90^\circ$ ,  $\gamma = 120$ , with an hexagonal space group P6<sub>1</sub>22 and 2 molecules per asymmetric unit.

25 34. A crystallized polypeptide comprising (1) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (2) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (3) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ  
30 ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*; wherein the crystal has a P6<sub>1</sub>22 space group.

35. A crystallized polypeptide comprising a structure of a polypeptide that is defined by a substantial portion of the atomic coordinates set forth in FIGURE 9.

36. A method for determining the crystal structure of a homolog of a polypeptide, the method comprising:

5 (a) providing the three dimensional structure of a first crystallized polypeptide comprising (1) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (2) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (3) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a  
10 10 polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*;

15 (b) obtaining crystals of a second polypeptide comprising an amino acid sequence that is at least 70% identical to the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4, such that the three dimensional structure of the second crystallized polypeptide may be determined to a resolution of 3.5 Å or better; and

(c) determining the three dimensional structure of the second crystallized polypeptide by x-ray crystallography based on the atomic coordinates of the three dimensional structure provided in step (a).

37. The method of claim 36, wherein the atomic coordinates for the second  
20 crystallized polypeptide have a root mean square deviation from the backbone atoms of the first polypeptide of not more than 1.0 Å for all backbone atoms shared in common with the first polypeptide and the second polypeptide.

38. A method for homology modeling a homolog of peptidyl-tRNA hydrolase from *P. aeruginosa*, comprising:

25 (a) aligning the amino acid sequence of a homolog of peptidyl-tRNA hydrolase from *P. aeruginosa* with an amino acid sequence of SEQ ID NO: 2 or SEQ ID NO: 4 and incorporating the sequence of the homolog of peptidyl-tRNA hydrolase from *P. aeruginosa* into a model of peptidyl-tRNA hydrolase from *P. aeruginosa* derived from structure coordinates as listed in FIGURE 9 to yield a preliminary model of the homolog of peptidyl-tRNA hydrolase from *P. aeruginosa*;

30 (b) subjecting the preliminary model to energy minimization to yield an energy minimized model;

(c) remodeling regions of the energy minimized model where stereochemistry restraints are violated to yield a final model of the homolog of peptidyl-tRNA hydrolase from *P. aeruginosa*.

39. A method for obtaining structural information about a molecule or a molecular complex of unknown structure comprising:

(a) crystallizing the molecule or molecular complex;

(b) generating an x-ray diffraction pattern from the crystallized molecule or molecular complex;

(c) applying at least a portion of the structure coordinates set forth in FIGURE 9 to 10 the x-ray diffraction pattern to generate a three-dimensional electron density map of at least a portion of the molecule or molecular complex whose structure is unknown.

40. A method for attempting to make a crystallized complex comprising a polypeptide and a modulator having a molecular weight of less than 5 kDa, the method comprising:

15 (a) crystallizing a polypeptide comprising (1) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (2) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (3) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ 20 ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*; such that crystals of the crystallized polypeptide will diffract x-rays to a resolution of 5 Å or better; and

(b) soaking the crystals in a solution comprising a potential modulator having a molecular weight of less than 5 kDa.

25 41. A method for incorporating a potential modulator in a crystal of a polypeptide, comprising placing a crystal of peptidyl-tRNA hydrolase from *P. aeruginosa* having unit cell dimensions  $a = 63.702 \text{ \AA}$ ,  $b = 63.702 \text{ \AA}$ ,  $c = 154.844 \text{ \AA}$ ,  $\alpha = \beta = 90^\circ$ ,  $\gamma = 120^\circ$ , with an hexagonal space group P6<sub>1</sub>22 in a solution comprising the potential modulator.

30 42. A computer readable storage medium comprising digitally encoded structural data, wherein the data comprises structural coordinates as listed in FIGURE 9 for the backbone atoms of at least about six amino acid residues from a druggable region of peptidyl-tRNA hydrolase from *P. aeruginosa*.

43. A scalable three-dimensional configuration of points, at least a portion of the points derived from some or all of the structure coordinates as listed in FIGURE 9 for a plurality of amino acid residues from a druggable region of peptidyl-tRNA hydrolase from *P. aeruginosa*.

5 44. The scalable three-dimensional configuration of points of claim 43, wherein the structure coordinates as listed in FIGURE 9 for the backbone atoms of at least about five amino acid residues from a druggable region of peptidyl-tRNA hydrolase from *P. aeruginosa* are used to derive part or all of the portion of points.

10 45. The scalable three-dimensional configuration of points of claim 43, wherein the structure coordinates as listed in FIGURE 9 for the backbone and optionally the side chain atoms of at least about ten amino acid residues from a druggable region of peptidyl-tRNA hydrolase from *P. aeruginosa* are used to derive part or all of the portion of points.

15 46. The scalable three-dimensional configuration of points of claim 43, wherein the structure coordinates as listed in FIGURE 9 for the backbone atoms of at least about fifteen amino acid residues from a druggable region of peptidyl-tRNA hydrolase from *P. aeruginosa* are used to derive part or all of the portion of points.

47. The scalable three-dimensional configuration of points of claim 43, wherein substantially all of the points are derived from structure coordinates as listed in FIGURE 9.

20 48. The scalable three-dimensional configuration of points of claim 43, wherein the structure coordinates as listed in FIGURE 9 for the atoms of the amino acid residues from any of the above-described druggable regions of peptidyl-tRNA hydrolase from *P. aeruginosa* are used to derive part or all of the portion of points:

25 49. A scalable three-dimensional configuration of points, comprising points having a root mean square deviation of less than about 1.0 Å from the three dimensional coordinates as listed in FIGURE 9 for the backbone atoms of at least five amino acid residues, wherein the five amino acid residues are from a druggable region of peptidyl-tRNA hydrolase from *P. aeruginosa*.

30 50. The scalable three-dimensional configuration of points of claim 49, wherein any point-to-point distance, calculated from the three dimensional coordinates as listed in FIGURE 9, between one of the backbone atoms for one of the five amino acid residues and another backbone atom of a different one of the five amino acid residues is not more than about 10 Å.

51. A scalable three-dimensional configuration of points comprising points having a root mean square deviation of less than about 1.0 Å from the three dimensional coordinates as listed in FIGURE 9 for the atoms of the amino acid residues from any of the above-described druggable regions of peptidyl-tRNA hydrolase from *P. aeruginosa*:

5 52. A computer readable storage medium comprising digitally encoded structural data, wherein the data comprise the identity and three-dimensional coordinates as listed in FIGURE 9 for the atoms of the amino acid residues from any of the above-described druggable regions of peptidyl-tRNA hydrolase from *P. aeruginosa*:

10 53. A scalable three-dimensional configuration of points, wherein the points have a root mean square deviation of less than about 1.0 Å from the three dimensional coordinates as listed in FIGURE 9 for the atoms of the amino acid residues from any of the above-described druggable regions of peptidyl-tRNA hydrolase from *P. aeruginosa*, wherein up to one amino acid residue in each of the regions may have a conservative substitution thereof.

15 54. A scalable three-dimensional configuration of points derived from a druggable region of a polypeptide, wherein the points have a root mean square deviation of less than about 1.0 Å from the three dimensional coordinates as listed in FIGURE 9 for the backbone atoms of at least ten amino acid residues that participate in the intersubunit contacts of peptidyl-tRNA hydrolase from *P. aeruginosa*.

20 55. A computer-assisted method for identifying an inhibitor of the activity of peptidyl-tRNA hydrolase from *P. aeruginosa*, comprising:

(a) supplying a computer modeling application with a set of structure coordinates as listed in FIGURE 9 for the atoms of the amino acid residues from any of the above-described druggable regions of peptidyl-tRNA hydrolase from *P. aeruginosa* so as to define part or all of a molecule or complex;

25 (b) supplying the computer modeling application with a set of structure coordinates of a chemical entity; and

(c) determining whether the chemical entity is expected to bind to or interfere with the molecule or complex.

30 56. The method of claim 55, wherein determining whether the chemical entity is expected to bind to or interfere with the molecule or complex comprises performing a fitting operation between the chemical entity and a druggable region of the molecule or complex, followed by computationally analyzing the results of the fitting operation to quantify the association between the chemical entity and the druggable region.

57. The method of claim 55, further comprising screening a library of chemical entities.

58. A computer-assisted method for designing an inhibitor of peptidyl-tRNA hydrolase activity comprising:

5 (a) supplying a computer modeling application with a set of structure coordinates having a root mean square deviation of less than about 1.0 Å from the structure coordinates as listed in FIGURE 9 for the atoms of the amino acid residues from any of the above-described druggable regions of peptidyl-tRNA hydrolase from *P. aeruginosa* so as to define part or all of a molecule or complex;

10 (b) supplying the computer modeling application with a set of structure coordinates for a chemical entity;

(c) evaluating the potential binding interactions between the chemical entity and the molecule or complex;

15 (d) structurally modifying the chemical entity to yield a set of structure coordinates for a modified chemical entity; and

(e) determining whether the modified chemical entity is an inhibitor expected to bind to or interfere with the molecule or complex, wherein binding to or interfering with the molecule or molecular complex is indicative of potential inhibition of peptidyl-tRNA hydrolase activity.

20 59. The method of claim 58, wherein determining whether the modified chemical entity is an inhibitor expected to bind to or interfere with the molecule or complex comprises performing a fitting operation between the chemical entity and the molecule or complex, followed by computationally analyzing the results of the fitting operation to evaluate the association between the chemical entity and the molecule or complex.

25 60. The method of claim 58, wherein the set of structure coordinates for the chemical entity is obtained from a chemical library.

61. A computer-assisted method for designing an inhibitor of peptidyl-tRNA hydrolase activity *de novo* comprising:

30 (a) supplying a computer modeling application with a set of three-dimensional coordinates derived from the structure coordinates as listed in FIGURE 9 for the atoms of the amino acid residues from any of the above-described druggable regions of peptidyl-tRNA hydrolase from *P. aeruginosa* so as to define part or all of a molecule or complex;

(b) computationally building a chemical entity represented by a set of structure coordinates; and

(c) determining whether the chemical entity is an inhibitor expected to bind to or interfere with the molecule or complex, wherein binding to or interfering with the molecule or complex is indicative of potential inhibition of peptidyl-tRNA hydrolase activity.

62. The method of claim 61, wherein determining whether the chemical entity is an inhibitor expected to bind to or interfere with the molecule or complex comprises performing a fitting operation between the chemical entity and a druggable region of the molecule or complex, followed by computationally analyzing the results of the fitting operation to quantify the association between the chemical entity and the druggable region.

10 63. The method of any of claims 55, 58 or 61, further comprising supplying or synthesizing the potential inhibitor, then assaying the potential inhibitor to determine whether it inhibits peptidyl-tRNA hydrolase activity.

15 64. A method for identifying a potential modulator for the prevention or treatment of a *P. aeruginosa* related disease or disorder, the method comprising:

20 (a) providing the three dimensional structure of a crystallized polypeptide comprising: (1) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (2) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (3) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*;

25 (b) obtaining a potential modulator for the prevention or treatment of *P. aeruginosa* related disease or disorder based on the three dimensional structure of the crystallized polypeptide;

30 (c) contacting the potential modulator with a second polypeptide comprising: (i) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (ii) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (iii) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-

tRNA hydrolase from *P. aeruginosa*; which second polypeptide may optionally be the same as the crystallized polypeptide; and

5 (d) assaying the activity of the second polypeptide, wherein a change in the activity of the second polypeptide indicates that the compound may be useful for prevention or treatment of a *P. aeruginosa* related disease or disorder.

65. A method for designing a candidate modulator for screening for inhibitors of a polypeptide, the method comprising:

10 (a) providing the three dimensional structure of a druggable region of a polypeptide comprising (1) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (2) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (3) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*; and

15 (b) designing a candidate modulator based on the three dimensional structure of the druggable region of the polypeptide.

66. A method for identifying a potential modulator of a polypeptide from a database, the method comprising:

20 (a) providing the three-dimensional coordinates for a plurality of the amino acids of a polypeptide comprising (1) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (2) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (3) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*;

25 (b) identifying a druggable region of the polypeptide; and

(c) selecting from a database at least one potential modulator comprising three dimensional coordinates which indicate that the modulator may bind or interfere with the druggable region.

30 67. The method of claim 66, wherein the modulator is a small molecule.

68. A method for preparing a potential modulator of a druggable region contained in a polypeptide, the method comprising:

(a) using the atomic coordinates for the backbone atoms of at least about six amino acid residues from a polypeptide of SEQ ID NO: 4, with a  $\pm$  a root mean square deviation 5 from the backbone atoms of the amino acid residues of not more than 1.0 Å, to generate one or more three-dimensional structures of a molecule comprising a druggable region from the polypeptide;

(b) employing one or more of the three dimensional structures of the molecule to design or select a potential modulator of the druggable region; and

10 (c) synthesizing or obtaining the modulator.

69. An apparatus for determining whether a compound is a potential modulator of a polypeptide, the apparatus comprising:

(a) a memory that comprises:

(i) the three dimensional coordinates and identities of at least about fifteen 15 atoms from a druggable region of a polypeptide comprising (1) an amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; (2) an amino acid sequence having at least about 95% identity with the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4; or (3) an amino acid sequence encoded by a polynucleotide that hybridizes under stringent conditions to the complementary strand of a polynucleotide having SEQ ID NO: 1 or SEQ 20 ID NO: 3 and has at least one biological activity of peptidyl-tRNA hydrolase from *P. aeruginosa*;

(ii) executable instructions; and

(b) a processor that is capable of executing instructions to:

(i) receive three-dimensional structural information for a candidate 25 modulator;

(ii) determine if the three-dimensional structure of the candidate modulator is complementary to the three dimensional coordinates of the atoms from the druggable region; and

(iii) output the results of the determination.

30 70. A method for making an inhibitor of peptidyl-tRNA hydrolase activity, the method comprising chemically or enzymatically synthesizing a chemical entity to yield an

inhibitor of peptidyl-tRNA hydrolase activity, the chemical entity having been identified during a computer-assisted process comprising supplying a computer modeling application with a set of structure coordinates of a molecule or complex, the molecule or complex comprising at least a portion of at least one druggable region from peptidyl-tRNA hydrolase

5 from *P. aeruginosa*; supplying the computer modeling application with a set of structure coordinates of a chemical entity; and determining whether the chemical entity is expected to bind or to interfere with the molecule or complex at a druggable region, wherein binding to or interfering with the molecule or complex is indicative of potential inhibition of peptidyl-tRNA hydrolase activity.

10 71. A computer readable storage medium comprising digitally encoded data, wherein the data comprises structural coordinates for a druggable region that is structurally homologous to the structure coordinates as listed in FIGURE 9 for a druggable region of peptidyl-tRNA hydrolase from *P. aeruginosa*.

72. A computer readable storage medium comprising digitally encoded structural  
15 data, wherein the data comprise a majority of the three-dimensional structure coordinates as listed in FIGURE 9.

73. The computer readable storage medium of claim 72, further comprising the identity of the atoms for the majority of the three-dimensional structure coordinates as listed in FIGURE 9.

20 The computer readable storage medium of claim 72, wherein the data comprise substantially all of the three-dimensional structure coordinates as listed in FIGURE 9.

**FIGURE 1**

SEQ ID NO: 1

GTGACTGCCGTACAAC TGATCGTTGGCCTGGAAACCCGGGT CCTGAAT  
5 ACGACCAGACCCGGCATAAC GCGGGGGCCCTTTCGTTGAGCGCCTGGCGCAT  
GCC CAGGGCGTCAGCCTCGTGGCTGACCGCAAGTATT CGGCCTGGTCGGCAA  
GTT CAGCCACCAGGGCAAGGACGTT CGTCTGTTGATCCGACCACCTACATGAA  
CCG CAGCGGCCAGTCCGTGGCGCGCTGGCGGGATTCTTCCGGATCGCCCCGG  
ACGCCATCCTGGTGGCCCACGACGAAC TCGACATGCCCTGGCGTCGCCAAG  
10 CTCAAGACCGGGCGCGGACACGGCGGGACAACGGGTTGCGCGACATCATCGC  
CCAGCTCGCAATCAGAATTCTTCCATCGCCTGCGGCTTGCATCGGCATCC  
GGGGCACAGCAGCCTGGTTCCGGTACGTGCTCGGCCGCGCCCCCGCGAGCG  
AGCAGGAACTGCTCGACACCAGCATCGACTT CGCCCTGGCGTGCTGCCGAA  
ATGCTCGCCGGGGACTGGACCCGGCGATGCAGAAGCTGCACAGCCAGAAGGC  
15 CTGA

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**FIGURE 2**

SEQ ID NO: 2

VTAVQLIVGLGNPGPEYDQTRHNAGALFVERLAHAQGVSLVADRKYFGLV

5 GKFHQGKDVRLLIPTTYMNRSGQSVAALAGFFRIAPDAILVAHDELDMPGVAKL  
KTGGGHGGHNGLRDIIAQLGNQNSFHRLRLGIGHPGHSSLVSGYVLGRAPRSEQUEL  
LDTSIDFALGVLPEMPLAGDWTRAMQKLHSQKA

**FIGURE 3**

SEQ ID NO: 3

GTGACTGCCGTACAAC TGATCGTGGCCTGGAAACCCGGGT CCTGAAT  
5 ACGACCAGACCCGGCATAACGC GGGGGCCCTTT CGTTGAGCGCCTGGCGCAT  
GCC CAGGGCGTCAGCCTCGTGGCTGACCGCAAGTATT CGGCCTGGCGCAA  
GTT CAGCCACCAGGGCAAGGACGTT CGTCTGTTGATCCGACCACCTACATGAA  
CCGCAGCGGCCAGTCCGTGGCGCGCTGGCGGGATTCTCCGGATCGCCCCGG  
ACGCCATCCTGGTGGCCCACGACGA ACTCGACATGCCCTGGCGTCGCCAAG  
10 CTCAAGACCGGGCGCGGACACGGCGGGACAACGGGTTGCGCGACATCATCGC  
CCAGCTCGGCAATCAGAATTCTTCCATCGCCTGCGGCTTGGCATCGGCCATCC  
GGGGCACAGCAGCCTGGTTCCGGTTACGTGCTGGCCCGCCCCCGCGCAGCG  
AGCAGGA ACTGCTCGACACCA CGCATCGACTTCGCCCTCGCGTGCTGCCGAA  
ATGCTCGCCGGGGACTGGACCCGGCGATGCAGAAGCTGCACAGCCAGAAGGC  
15 CTGA

**FIGURE 4**

SEQ ID NO: 4

VTAVQLIVGLGNPGPEYDQTRHNAGALFVERLAHAQGVSLVADRKYFGLV  
5 GKFSHQGKDVRLLIPTTYMNRSGQSVAALAGFFRIAPDAILVAHDELDMPGVAKL  
KTGGGHGGHNGLIRDIAQLGNQNSFHRLRLGIGHPGHSSLVSGYVLGRAPRSEQEL  
LDTSIDFALGVLPEMPLAGDWTRAMQKLHSQKA

**FIGURE 5**

SEQ ID NO: 5

Forward PCR Primer

5 GCGGCGCCCATATGACTGCCGTACAAC TGATC

SEQ ID NO: 6

10

Reverse PCR Primer

GCGCGGATCCGGCCTCTGGCTGTGCAG

**FIGURE 6****TABLE 1: Amino Acid and Nucleic Acid Properties**

Melting temperature (°C) of SEQ ID NO: 5 (forward PCR primer)	62
Restriction enzyme for SEQ ID NO: 5 (forward PCR primer)	NdeI
Melting temperature (°C) of SEQ ID NO: 6 (reverse PCR primer)	60
Restriction enzyme for SEQ ID NO: 6 (reverse PCR primer)	BamHI
Number of nucleic acid residues in SEQ ID NO: 1	585
Number of amino acid residues in SEQ ID NO: 2	194
Number of different nucleic acid residues between SEQ ID NO: 1 and SEQ ID NO: 3	0
Number of different amino acid residues between SEQ ID NO: 2 and SEQ ID NO: 4	0
Calculated molecular weight of SEQ ID NO: 2 polypeptide (kDa)	20.804
Calculated pI of SEQ ID NO: 2 polypeptide	10
Solubility of SEQ ID NO: 4 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the N-terminus)	Approximately one third
Solubility of SEQ ID NO: 4 polypeptide, determined as described in EXAMPLE 2 (with the His tag at the C-terminus)	No detectable expression
Amount of purified polypeptide having SEQ ID NO: 4, prepared and purified as described in EXAMPLE 8 (mg/L of culture)	3.9
Amount of purified polypeptide having SEQ ID NO: 4 soluble in buffer, as described in EXAMPLE 8 (mg/ml of buffer)	11

FIGURE 7

TABLE 2: Bioinformatic Analyses

Protein annotation and gene designation, if any	peptidyl-tRNA hydrolase, <i>pth</i>
COG Category	translation, ribosomal structure and biogenesis
COG ID Number	COG0193
Is SEQ ID NO: 2 classified as an essential gene?	yes
Most closely related protein from PDB	peptidyl-tRNA hydrolase (2pth)
Source organism for closest PDB protein	<i>Escherichia coli</i>
e-value for closest PDB Protein	58
% Identity between SEQ ID NO: 2 and the closest protein from PDB	72
% Positives between SEQ ID NO: 2 and the closest protein from PDB	4.00E-60
Number of Protein Hits in the VGDB	13
Number of Microorganisms having VGDB Hits	13
Microorganisms having VGDB Hits <sup>1</sup>	[paer][himf][bsub][nmen][ecoli][spne][saur][efae][mgen][ctra][bbur][rpxx][h pyl]
First predicted epitopic region of SEQ ID NO: 2: rank score, amino acid residue numbers; amino acid sequence	1.188, 133->155, SEQ ID NO: 10: RLRLGIGHPGHSSLVSGYVLGRA
Second predicted epitopic region of SEQ ID NO: 2: rank score, amino acid residue numbers; amino acid sequence	1.187, 4->11, SEQ ID NO: 11: VQLIVGLG
Third predicted epitopic region of SEQ ID NO: 2: rank score, amino acid residue numbers; amino acid sequence	1.140, 24->44, SEQ ID NO: 12: AGALFVERLAHAQGVSLVADR

<sup>1</sup>Organisms are abbreviated as follows: ecoli = *Escherichia coli*; h pyl = *Helicobacter pylori*; paer = *Pseudomonas aeruginosa*; ctra = *Chlamydia trachomatis*; himf = *Haemophilus influenzae*; nmen = *Neisseria meningitidis*; rpxx = *Rickettsia prowazekii*; bbur = *Borrelia burgdorferi*; bsub = *Bacillus subtilis*; staph = *Staphylococcus aureus*; spne = *Streptococcus pneumoniae*; mgen = *Mycoplasma genitalium*; efae = *Enterococcus faecalis*.

FIGURE 8

TABLE 3: X-ray Structure Data

Data collection	
Wavelength	1.54
Space group	P6(1)22
Unit cell dimensions (Å)	$a = b = 63.702, c = 154.844;$ $\alpha = \beta = 90^\circ, \gamma = 120^\circ$
Resolution range (Å)	30-1.90
Completeness (%) <sup>a,b</sup>	99.7 (99.8)
$I/I_{\text{min}}$	9.4 (2.93)
$R_{\text{merge}}$	6.3 (27.8)
Number of reflections	
Total	152832
Unique	15497
Structure Refinement	
$R_{\text{cryst}}^d$	18.8
$R_{\text{free}}$	22.8
Number of atoms	
protein	1453
solvent	215
Average B-factors	
protein (Å <sup>2</sup> )	14.4
solvent (Å <sup>2</sup> )	27
R.M.S. Deviations from ideal	
Bond (Å)	0.0044
Angle (°)	1.14

<sup>a</sup>Number in parentheses is the statistic for highest resolution shell.<sup>b</sup> $I \geq \sigma I$ <sup>c</sup> $R_{\text{sym}} = \sum_h (\sum_j |I_{j,h} - \langle I_h \rangle| / \sum_j I_{j,h})$ , where h = set of Miller indices and j = set of observations of reflection h.<sup>d</sup> $R_{\text{cryst}} = \sum_{hkl} |F_o - F_d| / \sum_{hkl} |F_o|$

FIGURE 9

	ATOM	1	CB	THR	1A	-47.075	90.346	-66.456	1.00	13.53	C
	ATOM	2	OG1	THR	1A	-47.543	90.425	-67.808	1.00	16.07	O
5	ATOM	3	CG2	THR	1A	-47.599	91.530	-65.664	1.00	17.49	C
	ATOM	4	C	THR	1A	-45.020	89.060	-67.094	1.00	6.29	C
	ATOM	5	O	THR	1A	-44.698	89.061	-68.279	1.00	5.51	O
	ATOM	6	N	THR	1A	-44.960	91.498	-67.080	1.00	9.53	N
	ATOM	7	CA	THR	1A	-45.532	90.330	-66.426	1.00	10.29	C
10	ATOM	8	N	ILE	2A	-44.933	87.985	-66.320	1.00	3.48	N
	ATOM	9	CA	ILE	2A	-44.465	86.707	-66.840	1.00	3.08	C
	ATOM	10	CB	ILE	2A	-43.996	85.789	-65.691	1.00	2.74	C
	ATOM	11	CG2	ILE	2A	-43.755	84.373	-66.206	1.00	4.38	C
	ATOM	12	CG1	ILE	2A	-42.742	86.381	-65.052	1.00	3.38	C
15	ATOM	13	CD1	ILE	2A	-41.630	86.632	-66.037	1.00	10.37	C
	ATOM	14	C	ILE	2A	-45.562	86.003	-67.629	1.00	3.20	C
	ATOM	15	O	ILE	2A	-46.696	85.879	-67.156	1.00	3.27	O
	ATOM	16	N	LYS	3A	-45.212	85.544	-68.827	1.00	3.01	N
	ATOM	17	CA	LYS	3A	-46.149	84.845	-69.697	1.00	6.53	C
20	ATOM	18	CB	LYS	3A	-46.243	85.564	-71.047	1.00	11.47	C
	ATOM	19	CG	LYS	3A	-46.781	86.990	-70.947	1.00	18.25	C
	ATOM	20	CD	LYS	3A	-46.818	87.676	-72.309	1.00	23.70	C
	ATOM	21	CE	LYS	3A	-47.752	86.949	-73.262	1.00	28.19	C
	ATOM	22	NZ	LYS	3A	-49.130	86.845	-72.690	1.00	29.16	N
25	ATOM	23	C	LYS	3A	-45.758	83.381	-69.914	1.00	3.71	C
	ATOM	24	O	LYS	3A	-46.511	82.614	-70.510	1.00	1.00	O
	ATOM	25	N	LEU	4A	-44.582	82.997	-69.423	1.00	2.05	N
	ATOM	26	CA	LEU	4A	-44.104	81.627	-69.565	1.00	2.22	C
	ATOM	27	CB	LEU	4A	-43.363	81.459	-70.899	1.00	1.89	C
30	ATOM	28	CG	LEU	4A	-42.761	80.079	-71.176	1.00	3.80	C
	ATOM	29	CD1	LEU	4A	-43.881	79.048	-71.239	1.00	3.64	C
	ATOM	30	CD2	LEU	4A	-41.968	80.096	-72.485	1.00	1.51	C
	ATOM	31	C	LEU	4A	-43.188	81.198	-68.416	1.00	1.19	C
	ATOM	32	O	LEU	4A	-42.206	81.871	-68.092	1.00	1.00	O
35	ATOM	33	N	ILE	5A	-43.531	80.075	-67.794	1.00	1.87	N
	ATOM	34	CA	ILE	5A	-42.732	79.525	-66.712	1.00	1.00	C
	ATOM	35	CB	ILE	5A	-43.525	79.426	-65.392	1.00	1.00	C
	ATOM	36	CG2	ILE	5A	-42.635	78.793	-64.314	1.00	1.51	C
	ATOM	37	CG1	ILE	5A	-43.967	80.823	-64.944	1.00	2.97	C
40	ATOM	38	CD1	ILE	5A	-44.883	80.829	-63.726	1.00	3.09	C
	ATOM	39	C	ILE	5A	-42.287	78.134	-67.160	1.00	1.28	C
	ATOM	40	O	ILE	5A	-43.110	77.265	-67.452	1.00	1.00	O
	ATOM	41	N	VAL	6A	-40.974	77.946	-67.231	1.00	1.00	N
	ATOM	42	CA	VAL	6A	-40.392	76.688	-67.673	1.00	1.32	C
45	ATOM	43	CB	VAL	6A	-39.310	76.941	-68.752	1.00	2.99	C
	ATOM	44	CG1	VAL	6A	-38.905	75.628	-69.409	1.00	2.74	C
	ATOM	45	CG2	VAL	6A	-39.819	77.924	-69.788	1.00	3.16	C
	ATOM	46	C	VAL	6A	-39.753	75.938	-66.502	1.00	1.53	C
	ATOM	47	O	VAL	6A	-39.081	76.533	-65.663	1.00	2.65	O
50	ATOM	48	N	GLY	7A	-39.977	74.630	-66.457	1.00	1.74	N
	ATOM	49	CA	GLY	7A	-39.423	73.804	-65.400	1.00	1.00	C
	ATOM	50	C	GLY	7A	-38.565	72.741	-66.044	1.00	2.89	C
	ATOM	51	O	GLY	7A	-39.080	71.810	-66.670	1.00	1.00	O
	ATOM	52	N	LEU	8A	-37.253	72.893	-65.887	1.00	1.00	N
55	ATOM	53	CA	LEU	8A	-36.264	71.998	-66.479	1.00	1.00	C
	ATOM	54	CB	LEU	8A	-34.899	72.689	-66.490	1.00	1.52	C
	ATOM	55	CG	LEU	8A	-34.816	74.032	-67.223	1.00	3.81	C
	ATOM	56	CD1	LEU	8A	-33.363	74.505	-67.261	1.00	2.62	C
	ATOM	57	CD2	LEU	8A	-35.361	73.882	-68.639	1.00	4.14	C
60	ATOM	58	C	LEU	8A	-36.125	70.615	-65.850	1.00	1.31	C

FIGURE 9-1

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	ATOM	59	O	LEU	8A	-36.184	70.455	-64.628	1.00	1.71	O
	ATOM	60	N	ALA	9A	-35.920	69.618	-66.703	1.00	1.89	N
	ATOM	61	CA	ALA	9A	-35.751	68.245	-66.242	1.00	3.38	C
5	ATOM	62	CB	ALA	9A	-36.987	67.792	-65.507	1.00	6.64	C
	ATOM	63	C	ALA	9A	-35.491	67.317	-67.412	1.00	5.53	C
	ATOM	64	O	ALA	9A	-35.545	67.733	-68.569	1.00	7.86	O
	ATOM	65	N	ASN	10A	-35.187	66.064	-67.094	1.00	4.29	N
	ATOM	66	CA	ASN	10A	-34.960	65.042	-68.104	1.00	4.39	C
10	ATOM	67	CB	ASN	10A	-33.736	64.185	-67.763	1.00	3.09	C
	ATOM	68	CG	ASN	10A	-32.426	64.850	-68.148	1.00	3.78	C
	ATOM	69	OD1	ASN	10A	-32.187	65.144	-69.317	1.00	5.67	O
	ATOM	70	ND2	ASN	10A	-31.571	65.074	-67.167	1.00	6.15	N
	ATOM	71	C	ASN	10A	-36.216	64.174	-68.087	1.00	5.86	C
	ATOM	72	O	ASN	10A	-36.851	64.009	-67.048	1.00	4.43	O
15	ATOM	73	N	PRO	11A	-36.606	63.631	-69.245	1.00	6.76	N
	ATOM	74	CD	PRO	11A	-36.140	63.922	-70.613	1.00	7.66	C
	ATOM	75	CA	PRO	11A	-37.804	62.795	-69.265	1.00	7.50	C
	ATOM	76	CB	PRO	11A	-38.319	62.992	-70.678	1.00	8.92	C
	ATOM	77	CG	PRO	11A	-37.051	63.050	-71.465	1.00	7.92	C
20	ATOM	78	C	PRO	11A	-37.446	61.342	-68.976	1.00	6.42	C
	ATOM	79	O	PRO	11A	-36.296	60.947	-69.134	1.00	7.32	O
	ATOM	80	N	GLY	12A	-38.432	60.566	-68.548	1.00	7.68	N
	ATOM	81	CA	GLY	12A	-38.196	59.162	-68.268	1.00	7.99	C
	ATOM	82	C	GLY	12A	-38.285	58.776	-66.806	1.00	7.65	C
25	ATOM	83	O	GLY	12A	-37.994	59.578	-65.915	1.00	6.95	O
	ATOM	84	N	PRO	13A	-38.679	57.525	-66.531	1.00	7.42	N
	ATOM	85	CD	PRO	13A	-39.200	56.549	-67.505	1.00	8.54	C
	ATOM	86	CA	PRO	13A	-38.809	57.017	-65.166	1.00	7.67	C
	ATOM	87	CB	PRO	13A	-39.275	55.579	-65.382	1.00	6.90	C
30	ATOM	88	CG	PRO	13A	-40.095	55.685	-66.646	1.00	4.45	C
	ATOM	89	C	PRO	13A	-37.514	57.095	-64.362	1.00	8.84	C
	ATOM	90	O	PRO	13A	-37.527	57.459	-63.187	1.00	9.19	O
	ATOM	91	N	GLU	14A	-36.397	56.771	-65.003	1.00	10.22	N
	ATOM	92	CA	GLU	14A	-35.109	56.782	-64.315	1.00	11.61	C
35	ATOM	93	CB	GLU	14A	-34.081	55.988	-65.123	1.00	15.70	C
	ATOM	94	CG	GLU	14A	-34.530	54.564	-65.468	1.00	25.30	C
	ATOM	95	CD	GLU	14A	-34.849	53.715	-64.242	1.00	30.12	C
	ATOM	96	OE1	GLU	14A	-35.781	54.073	-63.488	1.00	33.10	O
	ATOM	97	OE2	GLU	14A	-34.169	52.685	-64.035	1.00	32.34	O
40	ATOM	98	C	GLU	14A	-34.555	58.169	-63.993	1.00	8.01	C
	ATOM	99	O	GLU	14A	-33.566	58.291	-63.273	1.00	7.91	O
	ATOM	100	N	TYR	15A	-35.191	59.213	-64.511	1.00	6.95	N
	ATOM	101	CA	TYR	15A	-34.731	60.572	-64.241	1.00	6.80	C
	ATOM	102	CB	TYR	15A	-34.606	61.350	-65.554	1.00	6.12	C
45	ATOM	103	CG	TYR	15A	-33.490	60.872	-66.455	1.00	5.11	C
	ATOM	104	CD1	TYR	15A	-32.166	61.199	-66.184	1.00	6.58	C
	ATOM	105	CE1	TYR	15A	-31.134	60.753	-67.004	1.00	8.12	C
	ATOM	106	CD2	TYR	15A	-33.760	60.084	-67.577	1.00	6.92	C
	ATOM	107	CE2	TYR	15A	-32.733	59.632	-68.406	1.00	7.63	C
50	ATOM	108	CZ	TYR	15A	-31.424	59.969	-68.112	1.00	8.00	C
	ATOM	109	OH	TYR	15A	-30.400	59.517	-68.914	1.00	9.35	O
	ATOM	110	C	TYR	15A	-35.702	61.303	-63.320	1.00	8.78	C
	ATOM	111	O	TYR	15A	-35.325	62.231	-62.614	1.00	9.47	O
	ATOM	112	N	ASP	16A	-36.952	60.859	-63.331	1.00	9.97	N
55	ATOM	113	CA	ASP	16A	-38.017	61.475	-62.550	1.00	11.63	C
	ATOM	114	CB	ASP	16A	-39.061	60.424	-62.172	1.00	11.87	C
	ATOM	115	CG	ASP	16A	-40.354	61.047	-61.695	1.00	7.19	C
	ATOM	116	OD1	ASP	16A	-41.223	60.313	-61.197	1.00	11.02	O
	ATOM	117	OD2	ASP	16A	-40.504	62.277	-61.827	1.00	11.93	O
60	ATOM	118	C	ASP	16A	-37.630	62.279	-61.303	1.00	12.31	C
	ATOM	119	O	ASP	16A	-37.500	63.500	-61.368	1.00	15.69	O

FIGURE 9 - 2

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	ATOM	120	N	GLN	17A	-37.438	61.600	-60.176	1.00	12.79	N
	ATOM	121	CA	GLN	17A	-37.115	62.278	-58.921	1.00	10.92	C
	ATOM	122	CB	GLN	17A	-37.626	61.441	-57.743	1.00	14.22	C
	ATOM	123	CG	GLN	17A	-39.093	61.024	-57.853	1.00	17.50	C
5	ATOM	124	CD	GLN	17A	-40.049	62.206	-57.882	1.00	21.87	C
	ATOM	125	OE1	GLN	17A	-40.107	63.001	-56.941	1.00	25.25	O
	ATOM	126	NE2	GLN	17A	-40.807	62.326	-58.965	1.00	21.57	N
	ATOM	127	C	GLN	17A	-35.642	62.632	-58.687	1.00	9.05	C
	ATOM	128	O	GLN	17A	-35.199	62.719	-57.541	1.00	7.82	O
10	ATOM	129	N	THR	18A	-34.886	62.849	-59.757	1.00	5.77	N
	ATOM	130	CA	THR	18A	-33.474	63.194	-59.598	1.00	3.88	C
	ATOM	131	CB	THR	18A	-32.654	62.797	-60.840	1.00	4.24	C
	ATOM	132	OG1	THR	18A	-33.177	63.461	-61.994	1.00	3.19	O
	ATOM	133	CG2	THR	18A	-32.716	61.285	-61.060	1.00	3.10	C
15	ATOM	134	C	THR	18A	-33.320	64.690	-59.331	1.00	3.07	C
	ATOM	135	O	THR	18A	-34.208	65.486	-59.644	1.00	1.17	O
	ATOM	136	N	ARG	19A	-32.194	65.070	-58.737	1.00	1.34	N
	ATOM	137	CA	ARG	19A	-31.953	66.462	-58.407	1.00	2.22	C
	ATOM	138	CB	ARG	19A	-30.561	66.621	-57.778	1.00	1.00	C
20	ATOM	139	CG	ARG	19A	-30.324	65.848	-56.486	1.00	1.00	C
	ATOM	140	CD	ARG	19A	-28.941	66.210	-55.925	1.00	2.02	C
	ATOM	141	NE	ARG	19A	-28.664	65.617	-54.620	1.00	3.66	N
	ATOM	142	CZ	ARG	19A	-28.011	64.475	-54.427	1.00	5.49	C
	ATOM	143	NH1	ARG	19A	-27.554	63.777	-55.460	1.00	8.27	N
25	ATOM	144	NH2	ARG	19A	-27.796	64.036	-53.194	1.00	4.56	N
	ATOM	145	C	ARG	19A	-32.056	67.394	-59.613	1.00	1.00	C
	ATOM	146	O	ARG	19A	-32.522	68.527	-59.488	1.00	3.32	O
	ATOM	147	N	HIS	20A	-31.612	66.913	-60.771	1.00	1.00	N
30	ATOM	148	CA	HIS	20A	-31.604	67.711	-61.993	1.00	1.00	C
	ATOM	149	CB	HIS	20A	-30.816	66.969	-63.078	1.00	2.69	C
	ATOM	150	CG	HIS	20A	-30.099	67.872	-64.035	1.00	4.77	C
	ATOM	151	CD2	HIS	20A	-29.871	67.763	-65.364	1.00	5.27	C
	ATOM	152	ND1	HIS	20A	-29.462	69.027	-63.632	1.00	4.39	N
	ATOM	153	CE1	HIS	20A	-28.872	69.587	-64.672	1.00	4.22	C
35	ATOM	154	NE2	HIS	20A	-29.104	68.840	-65.737	1.00	3.23	N
	ATOM	155	C	HIS	20A	-33.003	68.073	-62.488	1.00	1.84	C
	ATOM	156	O	HIS	20A	-33.145	68.889	-63.397	1.00	1.00	O
	ATOM	157	N	ASN	21A	-34.029	67.471	-61.880	1.00	1.00	N
	ATOM	158	CA	ASN	21A	-35.419	67.739	-62.242	1.00	1.72	C
40	ATOM	159	CB	ASN	21A	-36.221	66.431	-62.289	1.00	1.00	C
	ATOM	160	CG	ASN	21A	-36.039	65.691	-63.589	1.00	1.00	C
	ATOM	161	OD1	ASN	21A	-34.979	65.764	-64.211	1.00	1.00	O
	ATOM	162	ND2	ASN	21A	-37.074	64.967	-64.009	1.00	2.66	N
	ATOM	163	C	ASN	21A	-36.099	68.703	-61.277	1.00	1.68	C
45	ATOM	164	O	ASN	21A	-37.324	68.777	-61.231	1.00	1.00	O
	ATOM	165	N	ALA	22A	-35.308	69.436	-60.498	1.00	1.00	N
	ATOM	166	CA	ALA	22A	-35.869	70.382	-59.543	1.00	1.96	C
	ATOM	167	CB	ALA	22A	-34.749	71.161	-58.859	1.00	5.70	C
	ATOM	168	C	ALA	22A	-36.840	71.347	-60.220	1.00	1.00	C
50	ATOM	169	O	ALA	22A	-37.944	71.588	-59.719	1.00	2.92	O
	ATOM	170	N	GLY	23A	-36.424	71.896	-61.358	1.00	1.00	N
	ATOM	171	CA	GLY	23A	-37.255	72.840	-62.086	1.00	1.45	C
	ATOM	172	C	GLY	23A	-38.602	72.271	-62.484	1.00	1.00	C
	ATOM	173	O	GLY	23A	-39.636	72.912	-62.298	1.00	1.00	O
55	ATOM	174	N	ALA	24A	-38.597	71.060	-63.033	1.00	1.00	N
	ATOM	175	CA	ALA	24A	-39.841	70.428	-63.450	1.00	1.00	C
	ATOM	176	CB	ALA	24A	-39.551	69.101	-64.151	1.00	1.68	C
	ATOM	177	C	ALA	24A	-40.761	70.194	-62.261	1.00	2.85	C
	ATOM	178	O	ALA	24A	-41.986	70.287	-62.390	1.00	1.00	O
60	ATOM	179	N	LEU	25A	-40.176	69.883	-61.108	1.00	1.82	N
	ATOM	180	CA	LEU	25A	-40.964	69.641	-59.906	1.00	2.74	C

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	ATOM	181	CB	LEU	25A	-40.080	69.067	-58.787	1.00	2.83	C
	ATOM	182	CG	LEU	25A	-40.781	68.747	-57.462	1.00	1.89	C
	ATOM	183	CD1	LEU	25A	-41.790	67.626	-57.665	1.00	2.65	C
5	ATOM	184	CD2	LEU	25A	-39.741	68.332	-56.424	1.00	2.70	C
	ATOM	185	C	LEU	25A	-41.622	70.943	-59.459	1.00	3.26	C
	ATOM	186	O	LEU	25A	-42.772	70.950	-59.025	1.00	1.00	O
	ATOM	187	N	PHE	26A	-40.898	72.052	-59.565	1.00	1.77	N
	ATOM	188	CA	PHE	26A	-41.480	73.322	-59.181	1.00	1.86	C
	ATOM	189	CB	PHE	26A	-40.483	74.466	-59.356	1.00	1.00	C
10	ATOM	190	CG	PHE	26A	-41.107	75.816	-59.231	1.00	2.24	C
	ATOM	191	CD1	PHE	26A	-41.443	76.325	-57.984	1.00	1.24	C
	ATOM	192	CD2	PHE	26A	-41.406	76.561	-60.365	1.00	1.00	C
	ATOM	193	CE1	PHE	26A	-42.075	77.562	-57.868	1.00	1.00	C
15	ATOM	194	CE2	PHE	26A	-42.038	77.796	-60.256	1.00	1.33	C
	ATOM	195	CZ	PHE	26A	-42.373	78.293	-59.005	1.00	1.00	C
	ATOM	196	C	PHE	26A	-42.707	73.608	-60.036	1.00	1.57	C
	ATOM	197	O	PHE	26A	-43.740	74.011	-59.518	1.00	1.52	O
	ATOM	198	N	VAL	27A	-42.593	73.402	-61.345	1.00	1.15	N
20	ATOM	199	CA	VAL	27A	-43.717	73.659	-62.242	1.00	1.00	C
	ATOM	200	CB	VAL	27A	-43.254	73.690	-63.726	1.00	1.00	C
	ATOM	201	CG1	VAL	27A	-44.455	73.881	-64.655	1.00	5.73	C
	ATOM	202	CG2	VAL	27A	-42.273	74.835	-63.931	1.00	2.04	C
	ATOM	203	C	VAL	27A	-44.877	72.675	-62.078	1.00	1.68	C
25	ATOM	204	O	VAL	27A	-46.034	73.036	-62.305	1.00	1.00	O
	ATOM	205	N	GLU	28A	-44.578	71.441	-61.681	1.00	1.07	N
	ATOM	206	CA	GLU	28A	-45.636	70.457	-61.478	1.00	1.48	C
	ATOM	207	CB	GLU	28A	-45.044	69.060	-61.256	1.00	1.00	C
	ATOM	208	CG	GLU	28A	-46.070	67.954	-61.396	1.00	7.79	C
	ATOM	209	CD	GLU	28A	-45.501	66.581	-61.119	1.00	4.86	C
30	ATOM	210	OE1	GLU	28A	-44.369	66.295	-61.558	1.00	7.74	O
	ATOM	211	OE2	GLU	28A	-46.200	65.786	-60.462	1.00	9.93	O
	ATOM	212	C	GLU	28A	-46.466	70.880	-60.260	1.00	2.25	C
	ATOM	213	O	GLU	28A	-47.691	70.748	-60.254	1.00	3.18	O
35	ATOM	214	N	ARG	29A	-45.790	71.391	-59.233	1.00	1.00	N
	ATOM	215	CA	ARG	29A	-46.461	71.849	-58.018	1.00	2.69	C
	ATOM	216	CB	ARG	29A	-45.440	72.190	-56.929	1.00	6.04	C
	ATOM	217	CG	ARG	29A	-44.671	71.006	-56.381	1.00	7.97	C
	ATOM	218	CD	ARG	29A	-45.556	70.082	-55.556	1.00	11.91	C
40	ATOM	219	NE	ARG	29A	-44.765	69.065	-54.869	1.00	13.66	N
	ATOM	220	CZ	ARG	29A	-45.251	68.196	-53.986	1.00	13.68	C
	ATOM	221	NH1	ARG	29A	-46.539	68.206	-53.672	1.00	15.72	N
	ATOM	222	NH2	ARG	29A	-44.439	67.325	-53.405	1.00	15.39	N
	ATOM	223	C	ARG	29A	-47.277	73.092	-58.323	1.00	2.80	C
	ATOM	224	O	ARG	29A	-48.419	73.222	-57.881	1.00	2.22	O
45	ATOM	225	N	LEU	30A	-46.671	74.009	-59.069	1.00	1.00	N
	ATOM	226	CA	LEU	30A	-47.333	75.245	-59.459	1.00	1.44	C
	ATOM	227	CB	LEU	30A	-46.381	76.105	-60.302	1.00	2.02	C
	ATOM	228	CG	LEU	30A	-46.840	77.510	-60.730	1.00	1.00	C
	ATOM	229	CD1	LEU	30A	-45.684	78.231	-61.413	1.00	6.09	C
50	ATOM	230	CD2	LEU	30A	-48.021	77.413	-61.673	1.00	5.54	C
	ATOM	231	C	LEU	30A	-48.589	74.915	-60.263	1.00	2.84	C
	ATOM	232	O	LEU	30A	-49.661	75.483	-60.027	1.00	2.47	O
	ATOM	233	N	ALA	31A	-48.455	73.989	-61.208	1.00	1.89	N
	ATOM	234	CA	ALA	31A	-49.584	73.604	-62.048	1.00	2.26	C
55	ATOM	235	CB	ALA	31A	-49.135	72.612	-63.112	1.00	2.51	C
	ATOM	236	C	ALA	31A	-50.721	73.008	-61.219	1.00	1.67	C
	ATOM	237	O	ALA	31A	-51.887	73.335	-61.427	1.00	1.00	O
	ATOM	238	N	HIS	32A	-50.375	72.147	-60.270	1.00	1.00	N
	ATOM	239	CA	HIS	32A	-51.381	71.516	-59.424	1.00	3.65	C
60	ATOM	240	CB	HIS	32A	-50.750	70.417	-58.561	1.00	7.40	C
	ATOM	241	CG	HIS	32A	-51.750	69.634	-57.774	1.00	10.18	C

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	ATOM	242	CD2	HIS	32A	-51.962	69.541	-56.440	1.00	12.23	C
	ATOM	243	ND1	HIS	32A	-52.724	68.864	-58.371	1.00	12.96	N
	ATOM	244	CE1	HIS	32A	-53.494	68.330	-57.440	1.00	13.57	C
	ATOM	245	NE2	HIS	32A	-53.053	68.726	-56.259	1.00	14.32	N
5	ATOM	246	C	HIS	32A	-52.066	72.541	-58.524	1.00	5.16	C
	ATOM	247	O	HIS	32A	-53.285	72.504	-58.341	1.00	3.04	O
	ATOM	248	N	ALA	33A	-51.273	73.454	-57.971	1.00	5.26	N
	ATOM	249	CA	ALA	33A	-51.796	74.496	-57.094	1.00	4.69	C
	ATOM	250	CB	ALA	33A	-50.637	75.287	-56.473	1.00	2.35	C
	ATOM	251	C	ALA	33A	-52.737	75.436	-57.849	1.00	5.73	C
10	ATOM	252	O	ALA	33A	-53.690	75.963	-57.270	1.00	4.91	O
	ATOM	253	N	GLN	34A	-52.482	75.629	-59.141	1.00	6.05	N
	ATOM	254	CA	GLN	34A	-53.308	76.514	-59.959	1.00	7.15	C
	ATOM	255	CB	GLN	34A	-52.420	77.326	-60.919	1.00	6.75	C
	ATOM	256	CG	GLN	34A	-51.403	78.229	-60.235	1.00	10.76	C
15	ATOM	257	CD	GLN	34A	-52.056	79.284	-59.363	1.00	12.81	C
	ATOM	258	OE1	GLN	34A	-52.945	80.010	-59.808	1.00	14.82	O
	ATOM	259	NE2	GLN	34A	-51.614	79.376	-58.112	1.00	14.83	N
	ATOM	260	C	GLN	34A	-54.390	75.780	-60.759	1.00	6.33	C
	ATOM	261	O	GLN	34A	-55.068	76.386	-61.590	1.00	6.53	O
20	ATOM	262	N	GLY	35A	-54.540	74.481	-60.507	1.00	5.57	N
	ATOM	263	CA	GLY	35A	-55.539	73.680	-61.204	1.00	5.81	C
	ATOM	264	C	GLY	35A	-55.314	73.592	-62.706	1.00	7.61	C
	ATOM	265	O	GLY	35A	-56.259	73.418	-63.485	1.00	7.32	O
	ATOM	266	N	VAL	36A	-54.052	73.691	-63.112	1.00	5.36	N
25	ATOM	267	CA	VAL	36A	-53.685	73.635	-64.521	1.00	3.92	C
	ATOM	268	CB	VAL	36A	-52.515	74.598	-64.824	1.00	3.54	C
	ATOM	269	CG1	VAL	36A	-52.067	74.436	-66.277	1.00	4.84	C
	ATOM	270	CG2	VAL	36A	-52.929	76.033	-64.536	1.00	2.36	C
	ATOM	271	C	VAL	36A	-53.259	72.245	-64.980	1.00	3.98	C
30	ATOM	272	O	VAL	36A	-52.405	71.608	-64.358	1.00	2.89	O
	ATOM	273	N	SER	37A	-53.847	71.793	-56.081	1.00	3.52	N
	ATOM	274	CA	SER	37A	-53.512	70.494	-66.652	1.00	4.67	C
	ATOM	275	CB	SER	37A	-54.707	69.920	-67.424	1.00	5.75	C
	ATOM	276	OG	SER	37A	-55.760	69.528	-66.557	1.00	7.63	O
35	ATOM	277	C	SER	37A	-52.343	70.681	-67.614	1.00	3.19	C
	ATOM	278	O	SER	37A	-52.364	71.585	-68.445	1.00	4.82	O
	ATOM	279	N	LEU	38A	-51.322	69.838	-67.499	1.00	2.75	N
	ATOM	280	CA	LEU	38A	-50.177	69.927	-68.396	1.00	3.88	C
	ATOM	281	CB	LEU	38A	-48.880	69.593	-67.644	1.00	4.62	C
40	ATOM	282	CG	LEU	38A	-48.507	70.597	-66.544	1.00	5.58	C
	ATOM	283	CD1	LEU	38A	-47.239	70.148	-65.826	1.00	6.10	C
	ATOM	284	CD2	LEU	38A	-48.292	71.982	-67.165	1.00	5.95	C
	ATOM	285	C	LEU	38A	-50.401	68.952	-69.556	1.00	3.84	C
	ATOM	286	O	LEU	38A	-50.354	67.739	-69.378	1.00	3.27	O
45	ATOM	287	N	VAL	39A	-50.650	69.500	-70.741	1.00	4.59	N
	ATOM	288	CA	VAL	39A	-50.922	68.705	-71.933	1.00	4.03	C
	ATOM	289	CB	VAL	39A	-51.904	69.453	-72.865	1.00	5.84	C
	ATOM	290	CG1	VAL	39A	-52.140	68.643	-74.132	1.00	3.93	C
	ATOM	291	CG2	VAL	39A	-53.218	69.714	-72.141	1.00	4.78	C
50	ATOM	292	C	VAL	39A	-49.689	68.319	-72.751	1.00	3.73	C
	ATOM	293	O	VAL	39A	-48.865	69.166	-73.099	1.00	4.42	O
	ATOM	294	N	ALA	40A	-49.568	67.031	-73.058	1.00	3.86	N
	ATOM	295	CA	ALA	40A	-48.451	66.550	-73.859	1.00	2.21	C
	ATOM	296	CB	ALA	40A	-48.411	65.017	-73.856	1.00	3.56	C
55	ATOM	297	C	ALA	40A	-48.657	67.068	-75.272	1.00	4.75	C
	ATOM	298	O	ALA	40A	-49.532	66.586	-76.001	1.00	6.49	O
	ATOM	299	N	ASP	41A	-47.863	68.063	-75.654	1.00	4.30	N
	ATOM	300	CA	ASP	41A	-47.970	68.649	-76.977	1.00	4.66	C
	ATOM	301	CB	ASP	41A	-48.323	70.137	-76.872	1.00	2.84	C
60	ATOM	302	CG	ASP	41A	-48.863	70.693	-78.169	1.00	2.42	C

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	ATOM	303	OD1	ASP	41A	-48.415	70.242	-79.237	1.00	4.68	O
	ATOM	304	OD2	ASP	41A	-49.732	71.590	-78.126	1.00	3.54	O
	ATOM	305	C	ASP	41A	-46.655	68.500	-77.730	1.00	3.54	C
	ATOM	306	O	ASP	41A	-45.686	69.200	-77.449	1.00	3.14	O
5	ATOM	307	N	ARG	42A	-46.634	67.589	-78.693	1.00	4.75	N
	ATOM	308	CA	ARG	42A	-45.436	67.345	-79.479	1.00	3.76	C
	ATOM	309	CB	ARG	42A	-45.709	66.244	-80.502	1.00	4.61	C
	ATOM	310	CG	ARG	42A	-44.490	65.802	-81.283	1.00	6.73	C
	ATOM	311	CD	ARG	42A	-44.836	64.660	-82.239	1.00	6.84	C
	ATOM	312	NE	ARG	42A	-45.189	63.406	-81.570	1.00	7.48	N
10	ATOM	313	CZ	ARG	42A	-44.312	62.543	-81.061	1.00	6.12	C
	ATOM	314	NH1	ARG	42A	-43.008	62.789	-81.125	1.00	2.39	N
	ATOM	315	NH2	ARG	42A	-44.737	61.407	-80.521	1.00	4.79	N
	ATOM	316	C	ARG	42A	-44.966	68.613	-80.187	1.00	3.81	C
	ATOM	317	O	ARG	42A	-43.766	68.807	-80.393	1.00	3.12	O
	ATOM	318	N	LYS	43A	-45.906	69.486	-80.538	1.00	3.21	N
15	ATOM	319	CA	LYS	43A	-45.549	70.722	-81.227	1.00	2.31	C
	ATOM	320	CB	LYS	43A	-46.802	71.518	-81.602	1.00	3.51	C
	ATOM	321	CG	LYS	43A	-47.740	70.749	-82.527	1.00	5.83	C
	ATOM	322	CD	LYS	43A	-48.846	71.627	-83.102	1.00	10.95	C
	ATOM	323	CE	LYS	43A	-49.718	72.252	-82.020	1.00	12.62	C
	ATOM	324	NZ	LYS	43A	-49.009	73.316	-81.261	1.00	14.99	N
20	ATOM	325	C	LYS	43A	-44.592	71.602	-80.438	1.00	2.79	C
	ATOM	326	O	LYS	43A	-43.844	72.374	-81.028	1.00	4.45	O
	ATOM	327	N	TYR	44A	-44.607	71.497	-79.113	1.00	1.00	N
	ATOM	328	CA	TYR	44A	-43.697	72.308	-78.306	1.00	2.04	C
	ATOM	329	CB	TYR	44A	-44.488	73.213	-77.367	1.00	1.00	C
	ATOM	330	CG	TYR	44A	-45.523	74.024	-78.114	1.00	2.73	C
25	ATOM	331	CD1	TYR	44A	-46.879	73.736	-77.995	1.00	4.30	C
	ATOM	332	CE1	TYR	44A	-47.832	74.440	-78.723	1.00	5.05	C
	ATOM	333	CD2	TYR	44A	-45.137	75.043	-78.983	1.00	5.72	C
	ATOM	334	CE2	TYR	44A	-46.079	75.756	-79.720	1.00	7.11	C
	ATOM	335	CZ	TYR	44A	-47.425	75.450	-79.585	1.00	8.71	C
	ATOM	336	OH	TYR	44A	-48.362	76.168	-80.297	1.00	12.07	O
30	ATOM	337	C	TYR	44A	-42.711	71.450	-77.525	1.00	1.00	C
	ATOM	338	O	TYR	44A	-42.105	71.900	-76.557	1.00	1.08	O
	ATOM	339	N	PHE	45A	-42.567	70.203	-77.955	1.00	1.59	N
	ATOM	340	CA	PHE	45A	-41.618	69.279	-77.342	1.00	1.00	C
	ATOM	341	CB	PHE	45A	-40.208	69.675	-77.774	1.00	2.88	C
	ATOM	342	CG	PHE	45A	-40.068	69.877	-79.253	1.00	5.61	C
35	ATOM	343	CD1	PHE	45A	-40.115	68.793	-80.125	1.00	9.29	C
	ATOM	344	CD2	PHE	45A	-39.915	71.153	-79.782	1.00	7.48	C
	ATOM	345	CE1	PHE	45A	-40.011	68.976	-81.501	1.00	9.29	C
	ATOM	346	CE2	PHE	45A	-39.811	71.348	-81.159	1.00	8.29	C
	ATOM	347	CZ	PHE	45A	-39.860	70.255	-82.020	1.00	7.53	C
	ATOM	348	C	PHE	45A	-41.685	69.214	-75.820	1.00	1.00	C
40	ATOM	349	O	PHE	45A	-40.650	69.226	-75.149	1.00	1.00	O
	ATOM	350	N	GLY	46A	-42.890	69.144	-75.266	1.00	1.00	N
	ATOM	351	CA	GLY	46A	-42.997	69.070	-73.821	1.00	1.00	C
	ATOM	352	C	GLY	46A	-44.421	69.076	-73.316	1.00	1.01	C
	ATOM	353	O	GLY	46A	-45.357	68.859	-74.082	1.00	2.94	O
	ATOM	354	N	LEU	47A	-44.576	69.307	-72.016	1.00	2.40	N
45	ATOM	355	CA	LEU	47A	-45.888	69.365	-71.391	1.00	1.00	C
	ATOM	356	CB	LEU	47A	-45.860	68.695	-70.020	1.00	1.16	C
	ATOM	357	CG	LEU	47A	-45.365	67.246	-70.021	1.00	2.74	C
	ATOM	358	CD1	LEU	47A	-45.407	66.684	-68.612	1.00	4.58	C
	ATOM	359	CD2	LEU	47A	-46.236	66.414	-70.952	1.00	1.00	C
	ATOM	360	C	LEU	47A	-46.218	70.843	-71.248	1.00	1.26	C
50	ATOM	361	O	LEU	47A	-45.431	71.604	-70.680	1.00	1.00	O
	ATOM	362	N	VAL	48A	-47.379	71.236	-71.762	1.00	1.81	N
	ATOM	363	CA	VAL	48A	-47.807	72.635	-71.742	1.00	2.91	C

**FIGURE 9 - 6**

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	ATOM	364	CB	VAL	48A	-47.925	73.187	-73.186	1.00	5.03	C
	ATOM	365	CG1	VAL	48A	-46.620	72.969	-73.935	1.00	6.02	C
	ATOM	366	CG2	VAL	48A	-49.068	72.501	-73.915	1.00	10.10	C
	ATOM	367	C	VAL	48A	-49.140	72.885	-71.041	1.00	2.18	C
5	ATOM	368	O	VAL	48A	-50.125	72.187	-71.285	1.00	3.39	O
	ATOM	369	N	GLY	49A	-49.157	73.894	-70.174	1.00	2.60	N
	ATOM	370	CA	GLY	49A	-50.376	74.248	-69.463	1.00	4.21	C
	ATOM	371	C	GLY	49A	-50.638	75.740	-69.562	1.00	3.44	C
	ATOM	372	O	GLY	49A	-49.707	76.529	-69.735	1.00	2.81	O
10	ATOM	373	N	LYS	50A	-51.899	76.141	-69.440	1.00	4.77	N
	ATOM	374	CA	LYS	50A	-52.245	77.555	-69.538	1.00	6.93	C
	ATOM	375	CB	LYS	50A	-52.813	77.847	-70.932	1.00	10.17	C
	ATOM	376	CG	LYS	50A	-53.131	79.309	-71.197	1.00	16.75	C
	ATOM	377	CD	LYS	50A	-53.654	79.518	-72.617	1.00	20.36	C
15	ATOM	378	CE	LYS	50A	-55.002	78.842	-72.838	1.00	24.17	C
	ATOM	379	NZ	LYS	50A	-56.094	79.494	-72.060	1.00	25.42	N
	ATOM	380	C	LYS	50A	-53.252	78.004	-68.490	1.00	5.87	C
	ATOM	381	O	LYS	50A	-54.130	77.242	-68.095	1.00	3.41	O
	ATOM	382	N	PHE	51A	-53.118	79.250	-68.044	1.00	7.45	N
20	ATOM	383	CA	PHE	51A	-54.053	79.819	-67.082	1.00	7.81	C
	ATOM	384	CB	PHE	51A	-53.714	79.387	-65.647	1.00	10.96	C
	ATOM	385	CG	PHE	51A	-52.503	80.052	-65.059	1.00	13.30	C
	ATOM	386	CD1	PHE	51A	-52.621	81.249	-64.355	1.00	15.42	C
	ATOM	387	CD2	PHE	51A	-51.250	79.456	-65.161	1.00	15.06	C
25	ATOM	388	CE1	PHE	51A	-51.506	81.837	-63.756	1.00	16.08	C
	ATOM	389	CE2	PHE	51A	-50.131	80.034	-64.568	1.00	16.03	C
	ATOM	390	CZ	PHE	51A	-50.259	81.228	-63.861	1.00	17.18	C
	ATOM	391	C	PHE	51A	-54.031	81.336	-67.234	1.00	7.87	C
	ATOM	392	O	PHE	51A	-53.092	81.893	-67.803	1.00	8.80	O
30	ATOM	393	N	SER	52A	-55.074	81.994	-66.749	1.00	7.15	N
	ATOM	394	CA	SER	52A	-55.166	83.441	-66.871	1.00	6.48	C
	ATOM	395	CB	SER	52A	-56.603	83.845	-67.218	1.00	9.11	C
	ATOM	396	OG	SER	52A	-57.052	83.186	-68.389	1.00	13.76	O
	ATOM	397	C	SER	52A	-54.740	84.176	-65.614	1.00	5.40	C
35	ATOM	398	O	SER	52A	-54.953	83.701	-64.502	1.00	5.52	O
	ATOM	399	N	HIS	53A	-54.122	85.337	-65.804	1.00	6.43	N
	ATOM	400	CA	HIS	53A	-53.707	86.168	-64.688	1.00	3.93	C
	ATOM	401	CB	HIS	53A	-52.314	85.811	-64.180	1.00	4.24	C
	ATOM	402	CG	HIS	53A	-51.992	86.456	-62.870	1.00	6.40	C
40	ATOM	403	CD2	HIS	53A	-52.566	86.324	-61.649	1.00	3.62	C
	ATOM	404	ND1	HIS	53A	-51.012	87.414	-62.730	1.00	3.10	N
	ATOM	405	CE1	HIS	53A	-50.996	87.845	-61.482	1.00	3.97	C
	ATOM	406	NE2	HIS	53A	-51.929	87.200	-60.805	1.00	4.87	N
	ATOM	407	C	HIS	53A	-53.720	87.620	-65.110	1.00	5.30	C
45	ATOM	408	O	HIS	53A	-52.971	88.032	-66.001	1.00	2.27	O
	ATOM	409	N	GLN	54A	-54.586	88.386	-64.457	1.00	4.15	N
	ATOM	410	CA	GLN	54A	-54.741	89.799	-64.740	1.00	6.05	C
	ATOM	411	CB	GLN	54A	-53.488	90.570	-64.276	1.00	5.48	C
	ATOM	412	CG	GLN	54A	-53.287	90.479	-62.746	1.00	2.51	C
50	ATOM	413	CD	GLN	54A	-52.196	91.385	-62.184	1.00	6.58	C
	ATOM	414	OE1	GLN	54A	-52.023	91.473	-60.965	1.00	5.86	O
	ATOM	415	NE2	GLN	54A	-51.458	92.059	-63.060	1.00	5.07	N
	ATOM	416	C	GLN	54A	-55.044	90.028	-66.224	1.00	7.00	C
	ATOM	417	O	GLN	54A	-54.496	90.932	-66.860	1.00	7.88	O
55	ATOM	418	N	GLY	55A	-55.921	89.181	-66.760	1.00	6.86	N
	ATOM	419	CA	GLY	55A	-56.349	89.293	-68.145	1.00	8.85	C
	ATOM	420	C	GLY	55A	-55.434	88.773	-69.235	1.00	9.69	C
	ATOM	421	O	GLY	55A	-55.723	88.955	-70.414	1.00	9.96	O
	ATOM	422	N	ALA	56A	-54.335	88.129	-68.860	1.00	9.11	N
60	ATOM	423	CA	ALA	56A	-53.404	87.612	-69.854	1.00	8.72	C
	ATOM	424	CB	ALA	56A	-52.103	88.402	-69.820	1.00	8.99	C

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	ATOM	425	C	ALA	56A	-53.119	86.135	-69.639	1.00	9.60 C
	ATOM	426	O	ALA	56A	-53.235	85.620	-68.524	1.00	9.28 O
	ATOM	427	N	ASP	57A	-52.754	85.461	-70.725	1.00	8.89 N
	ATOM	428	CA	ASP	57A	-52.435	84.042	-70.688	1.00	8.00 C
5	ATOM	429	CB	ASP	57A	-52.532	83.438	-72.092	1.00	10.97 C
	ATOM	430	CG	ASP	57A	-53.954	83.331	-72.589	1.00	14.23 C
	ATOM	431	OD1	ASP	57A	-54.137	83.103	-73.803	1.00	17.50 O
	ATOM	432	OD2	ASP	57A	-54.885	83.457	-71.768	1.00	15.79 O
	ATOM	433	C	ASP	57A	-51.023	83.830	-70.170	1.00	5.94 C
	ATOM	434	O	ASP	57A	-50.082	84.480	-70.631	1.00	7.57 O
10	ATOM	435	N	VAL	58A	-50.892	82.937	-69.196	1.00	3.48 N
	ATOM	436	CA	VAL	58A	-49.598	82.575	-68.631	1.00	3.89 C
	ATOM	437	CB	VAL	58A	-49.552	82.833	-67.110	1.00	4.00 C
	ATOM	438	CG1	VAL	58A	-48.184	82.454	-66.550	1.00	4.48 C
	ATOM	439	CG2	VAL	58A	-49.831	84.313	-66.834	1.00	3.15 C
	ATOM	440	C	VAL	58A	-49.455	81.079	-68.907	1.00	4.13 C
15	ATOM	441	O	VAL	58A	-50.336	80.289	-68.559	1.00	5.53 O
	ATOM	442	N	ARG	59A	-48.359	80.686	-69.540	1.00	2.93 N
	ATOM	443	CA	ARG	59A	-48.151	79.277	-69.863	1.00	3.40 C
	ATOM	444	CB	ARG	59A	-47.866	79.124	-71.361	1.00	3.15 C
	ATOM	445	CG	ARG	59A	-49.086	79.427	-72.239	1.00	8.12 C
	ATOM	446	CD	ARG	59A	-48.850	79.097	-73.707	1.00	10.41 C
20	ATOM	447	NE	ARG	59A	-50.061	79.248	-74.515	1.00	14.03 N
	ATOM	448	CZ	ARG	59A	-50.678	80.405	-74.750	1.00	16.18 C
	ATOM	449	NH1	ARG	59A	-51.773	80.434	-75.497	1.00	17.25 C
	ATOM	450	NH2	ARG	59A	-50.204	81.536	-74.246	1.00	16.61 N
	ATOM	451	C	ARG	59A	-47.056	78.596	-69.050	1.00	2.36 C
	ATOM	452	O	ARG	59A	-46.051	79.210	-68.696	1.00	2.33 O
25	ATOM	453	N	LEU	60A	-47.288	77.326	-68.741	1.00	2.20 N
	ATOM	454	CA	LEU	60A	-46.345	76.508	-67.985	1.00	2.36 C
	ATOM	455	CB	LEU	60A	-47.054	75.796	-66.833	1.00	1.24 C
	ATOM	456	CG	LEU	60A	-47.845	76.682	-65.869	1.00	1.00 C
	ATOM	457	CD1	LEU	60A	-48.381	75.832	-64.741	1.00	3.70 C
	ATOM	458	CD2	LEU	60A	-46.944	77.782	-65.309	1.00	1.97 C
30	ATOM	459	C	LEU	60A	-45.780	75.476	-68.950	1.00	1.70 C
	ATOM	460	O	LEU	60A	-46.518	74.881	-69.734	1.00	2.01 O
	ATOM	461	N	LEU	61A	-44.476	75.256	-68.886	1.00	1.00 N
	ATOM	462	CA	LEU	61A	-43.842	74.315	-69.787	1.00	1.70 C
	ATOM	463	CB	LEU	61A	-43.191	75.082	-70.940	1.00	2.53 C
	ATOM	464	CG	LEU	61A	-42.246	74.326	-71.874	1.00	1.00 C
35	ATOM	465	CD1	LEU	61A	-43.024	73.279	-72.660	1.00	2.50 C
	ATOM	466	CD2	LEU	61A	-41.562	75.316	-72.827	1.00	4.69 C
	ATOM	467	C	LEU	61A	-42.801	73.422	-69.135	1.00	1.00 C
	ATOM	468	O	LEU	61A	-41.926	73.884	-68.408	1.00	1.00 O
	ATOM	469	N	ILE	62A	-42.922	72.125	-69.381	1.00	1.63 N
	ATOM	470	CA	ILE	62A	-41.940	71.178	-68.881	1.00	2.33 C
40	ATOM	471	CB	ILE	62A	-42.536	70.133	-67.912	1.00	2.79 C
	ATOM	472	CG2	ILE	62A	-41.476	69.067	-67.589	1.00	1.44 C
	ATOM	473	CG1	ILE	62A	-43.007	70.821	-66.630	1.00	4.70 C
	ATOM	474	CD1	ILE	62A	-43.537	69.867	-65.572	1.00	3.15 C
	ATOM	475	C	ILE	62A	-41.481	70.479	-70.140	1.00	3.04 C
	ATOM	476	O	ILE	62A	-42.198	69.643	-70.686	1.00	3.83 O
45	ATOM	477	N	PRO	63A	-40.303	70.858	-70.656	1.00	3.51 N
	ATOM	478	CD	PRO	63A	-39.399	71.924	-70.201	1.00	2.92 C
	ATOM	479	CA	PRO	63A	-39.787	70.226	-71.871	1.00	2.01 C
	ATOM	480	CB	PRO	63A	-38.440	70.913	-72.073	1.00	2.96 C
	ATOM	481	CG	PRO	63A	-38.642	72.250	-71.465	1.00	3.76 C
	ATOM	482	C	PRO	63A	-39.616	68.734	-71.636	1.00	2.67 C
50	ATOM	483	O	PRO	63A	-39.275	68.314	-70.528	1.00	2.30 O
	ATOM	484	N	THR	64A	-39.835	67.945	-72.682	1.00	1.29 N
	ATOM	485	CA	THR	64A	-39.692	66.500	-72.589	1.00	2.46 C
	ATOM	486	CB	THR	64A	-39.552	66.135	-72.881	1.00	2.20 C
	ATOM	487	CG	THR	64A	-39.414	65.765	-73.250	1.00	2.00 C
	ATOM	488	O	THR	64A	-39.275	65.400	-73.636	1.00	1.79 C

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	ATOM	486	CB	THR	64A	-40.997	65.779	-72.991	1.00	3.31	C
	ATOM	487	OG1	THR	64A	-41.372	66.160	-74.320	1.00	5.54	O
	ATOM	488	CG2	THR	64A	-42.117	66.156	-72.022	1.00	3.62	C
	ATOM	489	C	THR	64A	-38.543	66.038	-73.474	1.00	2.95	C
5	ATOM	490	O	THR	64A	-38.408	64.855	-73.785	1.00	1.35	O
	ATOM	491	N	THR	65A	-37.732	67.001	-73.895	1.00	2.35	N
	ATOM	492	CA	THR	65A	-36.552	66.720	-74.688	1.00	3.31	C
	ATOM	493	CB	THR	65A	-36.014	67.981	-75.387	1.00	1.69	C
	ATOM	494	OG1	THR	65A	-35.718	68.977	-74.395	1.00	3.42	O
	ATOM	495	CG2	THR	65A	-37.029	68.542	-76.371	1.00	1.00	C
10	ATOM	496	C	THR	65A	-35.576	66.404	-73.576	1.00	4.91	C
	ATOM	497	O	THR	65A	-35.908	66.541	-72.395	1.00	5.83	O
	ATOM	498	N	TYR	66A	-34.377	65.972	-73.922	1.00	8.39	N
	ATOM	499	CA	TYR	66A	-33.420	65.734	-72.868	1.00	8.97	C
	ATOM	500	CB	TYR	66A	-32.265	64.884	-73.379	1.00	10.94	C
	ATOM	501	CG	TYR	66A	-32.547	63.428	-73.105	1.00	13.20	C
15	ATOM	502	CD1	TYR	66A	-32.564	62.485	-74.129	1.00	12.97	C
	ATOM	503	CE1	TYR	66A	-32.875	61.153	-73.868	1.00	11.59	C
	ATOM	504	CD2	TYR	66A	-32.842	63.006	-71.809	1.00	15.12	C
	ATOM	505	CE2	TYR	66A	-33.148	61.684	-71.534	1.00	16.61	C
	ATOM	506	CZ	TYR	66A	-33.165	60.763	-72.564	1.00	15.75	C
	ATOM	507	OH	TYR	66A	-33.471	59.461	-72.272	1.00	14.48	O
20	ATOM	508	C	TYR	66A	-32.983	67.115	-72.419	1.00	6.85	C
	ATOM	509	O	TYR	66A	-33.195	68.084	-73.140	1.00	7.38	O
	ATOM	510	N	MET	67A	-32.411	67.217	-71.225	1.00	7.40	N
	ATOM	511	CA	MET	67A	-31.991	68.515	-70.706	1.00	7.99	C
	ATOM	512	CB	MET	67A	-31.209	68.340	-69.401	1.00	9.03	C
	ATOM	513	CG	MET	67A	-30.887	69.650	-68.687	1.00	5.11	C
25	ATOM	514	SD	MET	67A	-32.369	70.459	-68.017	1.00	11.05	S
	ATOM	515	CE	MET	67A	-32.474	69.674	-66.396	1.00	6.71	C
	ATOM	516	C	MET	67A	-31.151	69.332	-71.687	1.00	8.16	C
	ATOM	517	O	MET	67A	-31.331	70.547	-71.802	1.00	4.96	O
	ATOM	518	N	ASN	68A	-30.235	68.671	-72.392	1.00	9.23	N
	ATOM	519	CA	ASN	68A	-29.358	69.364	-73.338	1.00	11.14	C
30	ATOM	520	CB	ASN	68A	-28.192	68.446	-73.739	1.00	16.37	C
	ATOM	521	CG	ASN	68A	-28.638	67.039	-74.078	1.00	19.09	C
	ATOM	522	OD1	ASN	68A	-27.837	66.101	-74.054	1.00	22.31	O
	ATOM	523	ND2	ASN	68A	-29.912	66.881	-74.404	1.00	22.11	N
	ATOM	524	C	ASN	68A	-30.030	69.946	-74.589	1.00	9.74	C
	ATOM	525	O	ASN	68A	-29.389	70.662	-75.366	1.00	9.29	O
35	ATOM	526	N	ARG	69A	-31.319	69.663	-74.770	1.00	8.50	N
	ATOM	527	CA	ARG	69A	-32.061	70.176	-75.920	1.00	7.23	C
	ATOM	528	CB	ARG	69A	-32.507	69.018	-76.812	1.00	10.71	C
	ATOM	529	CG	ARG	69A	-31.351	68.250	-77.435	1.00	13.81	C
	ATOM	530	CD	ARG	69A	-30.673	69.057	-78.529	1.00	15.95	C
	ATOM	531	NE	ARG	69A	-29.421	68.432	-78.947	1.00	19.05	N
40	ATOM	532	CZ	ARG	69A	-28.663	68.862	-79.949	1.00	20.33	C
	ATOM	533	NH1	ARG	69A	-27.536	68.228	-80.247	1.00	19.01	N
	ATOM	534	NH2	ARG	69A	-29.037	69.918	-80.659	1.00	23.40	N
	ATOM	535	C	ARG	69A	-33.281	70.995	-75.490	1.00	6.80	C
	ATOM	536	O	ARG	69A	-34.140	71.326	-76.307	1.00	1.63	O
	ATOM	537	N	SER	70A	-33.332	71.322	-74.201	1.00	4.11	N
45	ATOM	538	CA	SER	70A	-34.431	72.093	-73.608	1.00	4.52	C
	ATOM	539	CB	SER	70A	-34.078	72.462	-72.162	1.00	5.63	C
	ATOM	540	OG	SER	70A	-33.981	71.315	-71.349	1.00	11.27	O
	ATOM	541	C	SER	70A	-34.792	73.374	-74.368	1.00	4.30	C
	ATOM	542	O	SER	70A	-35.961	73.777	-74.399	1.00	5.52	O
	ATOM	543	N	GLY	71A	-33.786	74.012	-74.963	1.00	3.88	N
50	ATOM	544	CA	GLY	71A	-34.005	75.253	-75.691	1.00	2.13	C
	ATOM	545	C	GLY	71A	-34.912	75.099	-76.897	1.00	2.45	C
	ATOM	546	O	GLY	71A	-35.530	76.055	-77.360	1.00	1.26	O

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	ATOM	547	N	GLN	72A	-34.971	73.880	-77.414	1.00	2.29	N
	ATOM	548	CA	GLN	72A	-35.802	73.556	-78.557	1.00	5.21	C
	ATOM	549	CB	GLN	72A	-35.507	72.110	-78.958	1.00	10.57	C
	ATOM	550	CG	GLN	72A	-36.235	71.574	-80.159	1.00	15.03	C
5	ATOM	551	CD	GLN	72A	-35.755	70.177	-80.499	1.00	18.58	C
	ATOM	552	OE1	GLN	72A	-34.558	69.958	-80.694	1.00	20.08	O
	ATOM	553	NE2	GLN	72A	-36.678	69.225	-80.561	1.00	18.74	N
	ATOM	554	C	GLN	72A	-37.268	73.716	-78.143	1.00	4.30	C
	ATOM	555	O	GLN	72A	-38.101	74.244	-78.893	1.00	1.00	O
	ATOM	556	N	SER	73A	-37.576	73.257	-76.934	1.00	2.01	N
10	ATOM	557	CA	SER	73A	-38.937	73.339	-76.414	1.00	2.93	C
	ATOM	558	CB	SER	73A	-39.057	72.492	-75.139	1.00	4.70	C
	ATOM	559	OG	SER	73A	-40.375	72.520	-74.623	1.00	2.80	O
	ATOM	560	C	SER	73A	-39.327	74.786	-76.120	1.00	2.58	C
	ATOM	561	O	SER	73A	-40.367	75.270	-76.575	1.00	1.00	O
15	ATOM	562	N	VAL	74A	-38.478	75.478	-75.370	1.00	1.77	N
	ATOM	563	CA	VAL	74A	-38.732	76.867	-75.009	1.00	2.80	C
	ATOM	564	CB	VAL	74A	-37.571	77.430	-74.160	1.00	2.28	C
	ATOM	565	CG1	VAL	74A	-37.823	78.886	-73.825	1.00	1.00	C
	ATOM	566	CG2	VAL	74A	-37.431	76.613	-72.870	1.00	1.62	C
20	ATOM	567	C	VAL	74A	-38.953	77.767	-76.225	1.00	2.25	C
	ATOM	568	O	VAL	74A	-39.902	78.554	-76.258	1.00	1.00	O
	ATOM	569	N	ALA	75A	-38.093	77.640	-77.230	1.00	2.77	N
	ATOM	570	CA	ALA	75A	-38.215	78.469	-78.425	1.00	3.35	C
	ATOM	571	CB	ALA	75A	-36.984	78.309	-79.299	1.00	1.00	C
25	ATOM	572	C	ALA	75A	-39.479	78.162	-79.232	1.00	1.73	C
	ATOM	573	O	ALA	75A	-40.117	79.070	-79.759	1.00	4.44	O
	ATOM	574	N	ALA	76A	-39.840	76.890	-79.332	1.00	3.55	N
	ATOM	575	CA	ALA	76A	-41.034	76.501	-80.077	1.00	2.93	C
	ATOM	576	CB	ALA	76A	-41.199	74.971	-80.052	1.00	1.00	C
30	ATOM	577	C	ALA	76A	-42.280	77.156	-79.491	1.00	3.63	C
	ATOM	578	O	ALA	76A	-43.140	77.650	-80.219	1.00	3.65	O
	ATOM	579	N	LEU	77A	-42.385	77.142	-78.168	1.00	4.48	N
	ATOM	580	CA	LEU	77A	-43.551	77.719	-77.517	1.00	5.34	C
	ATOM	581	CB	LEU	77A	-43.714	77.150	-76.103	1.00	6.17	C
35	ATOM	582	CG	LEU	77A	-44.995	77.554	-75.359	1.00	5.07	C
	ATOM	583	CD1	LEU	77A	-46.220	77.059	-76.122	1.00	6.80	C
	ATOM	584	CD2	LEU	77A	-44.982	76.956	-73.962	1.00	7.62	C
	ATOM	585	C	LEU	77A	-43.448	79.234	-77.460	1.00	5.10	C
	ATOM	586	O	LEU	77A	-44.408	79.942	-77.759	1.00	5.93	O
40	ATOM	587	N	ALA	78A	-42.275	79.728	-77.078	1.00	5.02	N
	ATOM	588	CA	ALA	78A	-42.058	81.164	-76.980	1.00	5.59	C
	ATOM	589	CB	ALA	78A	-40.630	81.438	-76.531	1.00	5.72	C
	ATOM	590	C	ALA	78A	-42.330	81.851	-78.316	1.00	6.96	C
	ATOM	591	O	ALA	78A	-43.078	82.834	-78.386	1.00	5.88	O
45	ATOM	592	N	GLY	79A	-41.726	81.327	-79.376	1.00	4.76	N
	ATOM	593	CA	GLY	79A	-41.906	81.921	-80.688	1.00	3.15	C
	ATOM	594	C	GLY	79A	-43.329	81.899	-81.201	1.00	2.65	C
	ATOM	595	O	GLY	79A	-43.815	82.887	-81.766	1.00	2.74	O
	ATOM	596	N	PHE	80A	-44.001	80.774	-80.999	1.00	2.79	N
50	ATOM	597	CA	PHE	80A	-45.376	80.593	-81.454	1.00	2.15	C
	ATOM	598	CB	PHE	80A	-45.870	79.196	-81.073	1.00	3.34	C
	ATOM	599	CG	PHE	80A	-47.173	78.817	-81.718	1.00	3.75	C
	ATOM	600	CD1	PHE	80A	-47.188	78.149	-82.940	1.00	4.24	C
	ATOM	601	CD2	PHE	80A	-48.385	79.140	-81.111	1.00	5.10	C
55	ATOM	602	CE1	PHE	80A	-48.393	77.806	-83.552	1.00	5.43	C
	ATOM	603	CE2	PHE	80A	-49.599	78.802	-81.713	1.00	5.78	C
	ATOM	604	CZ	PHE	80A	-49.603	78.133	-82.935	1.00	6.24	C
	ATOM	605	C	PHE	80A	-46.328	81.641	-80.881	1.00	2.81	C
	ATOM	606	O	PHE	80A	-47.224	82.118	-81.577	1.00	2.29	O
60	ATOM	607	N	PHE	81A	-46.134	81.993	-79.613	1.00	3.22	N

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	ATOM	608	CA	PHE	81A	-46.986	82.985	-78.959	1.00	5.90	C
	ATOM	609	CB	PHE	81A	-47.438	82.458	-77.591	1.00	4.24	C
	ATOM	610	CG	PHE	81A	-48.372	81.285	-77.686	1.00	6.23	C
	ATOM	611	CD1	PHE	81A	-49.632	81.433	-78.256	1.00	7.74	C
5	ATOM	612	CD2	PHE	81A	-47.977	80.019	-77.260	1.00	6.43	C
	ATOM	613	CE1	PHE	81A	-50.484	80.343	-78.406	1.00	8.95	C
	ATOM	614	CE2	PHE	81A	-48.821	78.920	-77.407	1.00	7.65	C
	ATOM	615	CZ	PHE	81A	-50.079	79.082	-77.982	1.00	6.76	C
	ATOM	616	C	PHE	81A	-46.321	84.353	-78.815	1.00	7.85	C
	ATOM	617	O	PHE	81A	-46.822	85.221	-78.104	1.00	8.35	O
10	ATOM	618	N	ARG	82A	-45.196	84.532	-79.502	1.00	9.48	N
	ATOM	619	CA	ARG	82A	-44.444	85.784	-79.485	1.00	13.08	C
	ATOM	620	CB	ARG	82A	-45.196	86.862	-80.282	1.00	17.88	C
	ATOM	621	CG	ARG	82A	-45.189	86.625	-81.794	1.00	24.42	C
	ATOM	622	CD	ARG	82A	-45.897	85.328	-82.157	1.00	31.34	C
	ATOM	623	NE	ARG	82A	-45.614	84.884	-83.521	1.00	37.09	N
15	ATOM	624	CZ	ARG	82A	-46.002	85.522	-84.619	1.00	39.80	C
	ATOM	625	NH1	ARG	82A	-45.694	85.032	-85.812	1.00	41.45	N
	ATOM	626	NH2	ARG	82A	-46.699	86.647	-84.529	1.00	41.70	N
	ATOM	627	C	ARG	82A	-44.106	86.302	-78.086	1.00	12.16	C
	ATOM	628	O	ARG	82A	-44.303	87.480	-77.773	1.00	11.28	O
	ATOM	629	N	ILE	83A	-43.582	85.412	-77.251	1.00	9.21	C
20	ATOM	630	CA	ILE	83A	-43.197	85.770	-75.893	1.00	6.42	C
	ATOM	631	CB	ILE	83A	-43.472	84.593	-74.930	1.00	4.96	C
	ATOM	632	CG2	ILE	83A	-43.021	84.945	-73.512	1.00	3.65	C
	ATOM	633	CG1	ILE	83A	-44.964	84.244	-74.967	1.00	5.53	C
	ATOM	634	CD1	ILE	83A	-45.319	82.973	-74.218	1.00	4.43	C
	ATOM	635	C	ILE	83A	-41.707	86.131	-75.873	1.00	6.52	C
25	ATOM	636	O	ILE	83A	-40.860	85.332	-76.274	1.00	4.43	O
	ATOM	637	N	ALA	84A	-41.396	87.345	-75.423	1.00	5.32	N
	ATOM	638	CA	ALA	84A	-40.011	87.802	-75.361	1.00	4.65	C
	ATOM	639	CB	ALA	84A	-39.969	89.326	-75.336	1.00	6.43	C
	ATOM	640	C	ALA	84A	-39.297	87.241	-74.133	1.00	4.51	C
	ATOM	641	O	ALA	84A	-39.928	86.930	-73.131	1.00	4.29	O
30	ATOM	642	N	PRO	85A	-37.961	87.113	-74.197	1.00	4.93	N
	ATOM	643	CD	PRO	85A	-37.083	87.425	-75.340	1.00	5.99	C
	ATOM	644	CA	PRO	85A	-37.178	86.589	-73.075	1.00	5.44	C
	ATOM	645	CB	PRO	85A	-35.739	86.829	-73.525	1.00	7.50	C
	ATOM	646	CG	PRO	85A	-35.831	86.633	-75.008	1.00	6.24	C
	ATOM	647	C	PRO	85A	-37.486	87.259	-71.733	1.00	6.13	C
35	ATOM	648	O	PRO	85A	-37.449	86.606	-70.689	1.00	4.55	O
	ATOM	649	N	ASP	86A	-37.793	88.556	-71.758	1.00	3.98	N
	ATOM	650	CA	ASP	86A	-38.093	89.277	-70.523	1.00	5.85	C
	ATOM	651	CB	ASP	86A	-38.263	90.773	-70.794	1.00	8.63	C
	ATOM	652	CG	ASP	86A	-38.162	91.611	-69.531	1.00	14.35	C
	ATOM	653	OD1	ASP	86A	-38.734	92.722	-69.503	1.00	16.61	O
40	ATOM	654	OD2	ASP	86A	-37.495	91.175	-68.569	1.00	16.31	O
	ATOM	655	C	ASP	86A	-39.358	88.757	-69.847	1.00	3.15	C
	ATOM	656	O	ASP	86A	-39.566	88.985	-68.659	1.00	2.69	O
	ATOM	657	N	ALA	87A	-40.206	88.072	-70.605	1.00	3.41	N
	ATOM	658	CA	ALA	87A	-41.445	87.541	-70.047	1.00	3.21	C
	ATOM	659	CB	ALA	87A	-42.605	87.779	-71.030	1.00	3.94	C
45	ATOM	660	C	ALA	87A	-41.340	86.056	-69.701	1.00	2.99	C
	ATOM	661	O	ALA	87A	-42.348	85.405	-69.444	1.00	3.09	O
	ATOM	662	N	ILE	88A	-40.120	85.527	-69.689	1.00	2.69	N
	ATOM	663	CA	ILE	88A	-39.908	84.118	-69.372	1.00	2.29	C
	ATOM	664	CB	ILE	88A	-39.072	83.405	-70.460	1.00	1.74	C
	ATOM	665	CG2	ILE	88A	-38.926	81.926	-70.095	1.00	1.00	C
50	ATOM	666	CG1	ILE	88A	-39.727	83.579	-71.833	1.00	1.88	C
	ATOM	667	CD1	ILE	88A	-38.896	83.027	-72.989	1.00	2.05	C
	ATOM	668	C	ILE	88A	-39.172	83.903	-68.051	1.00	1.00	C
	ATOM	669	O	ILE	88A	-39.826	83.579	-71.833	1.00	1.88	O

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	ATOM	669	O	ILE	88A	-38.218	84.608	-67.737	1.00	4.48	O
	ATOM	670	N	LEU	89A	-39.630	82.924	-67.282	1.00	2.39	N
	ATOM	671	CA	LEU	89A	-39.002	82.554	-66.014	1.00	1.56	C
5	ATOM	672	CB	LEU	89A	-39.986	82.725	-64.848	1.00	3.17	C
	ATOM	673	CG	LEU	89A	-39.570	82.166	-63.483	1.00	2.35	C
	ATOM	674	CD1	LEU	89A	-38.304	82.856	-62.990	1.00	2.47	C
	ATOM	675	CD2	LEU	89A	-40.709	82.365	-62.483	1.00	3.18	C
	ATOM	676	C	LEU	89A	-38.638	81.084	-66.162	1.00	2.40	C
	ATOM	677	O	LEU	89A	-39.467	80.291	-66.597	1.00	1.00	O
10	ATOM	678	N	VAL	90A	-37.397	80.727	-65.836	1.00	1.68	N
	ATOM	679	CA	VAL	90A	-36.966	79.339	-65.936	1.00	1.00	C
	ATOM	680	CB	VAL	90A	-35.786	79.176	-66.925	1.00	2.68	C
	ATOM	681	CG1	VAL	90A	-35.411	77.706	-67.039	1.00	1.00	C
15	ATOM	682	CG2	VAL	90A	-36.152	79.738	-68.290	1.00	4.02	C
	ATOM	683	C	VAL	90A	-36.523	78.814	-64.575	1.00	1.00	C
	ATOM	684	O	VAL	90A	-35.617	79.363	-63.959	1.00	1.04	O
	ATOM	685	N	ALA	91A	-37.170	77.753	-64.108	1.00	1.18	N
	ATOM	686	CA	ALA	91A	-36.819	77.157	-62.829	1.00	2.29	C
	ATOM	687	CB	ALA	91A	-38.073	76.610	-62.123	1.00	1.00	C
20	ATOM	688	C	ALA	91A	-35.840	76.033	-63.101	1.00	1.00	C
	ATOM	689	O	ALA	91A	-36.057	75.198	-63.978	1.00	1.04	O
	ATOM	690	N	HIS	92A	-34.755	76.006	-62.344	1.00	1.00	N
	ATOM	691	CA	HIS	92A	-33.757	74.976	-62.542	1.00	2.87	C
25	ATOM	692	CB	HIS	92A	-32.738	75.429	-63.589	1.00	1.00	C
	ATOM	693	CG	HIS	92A	-31.843	76.535	-63.118	1.00	1.50	C
	ATOM	694	CD2	HIS	92A	-30.662	76.499	-62.457	1.00	1.00	C
	ATOM	695	ND1	HIS	92A	-32.154	77.869	-63.270	1.00	3.55	N
	ATOM	696	CE1	HIS	92A	-31.203	78.607	-62.724	1.00	1.00	C
30	ATOM	697	NB2	HIS	92A	-30.287	77.799	-62.223	1.00	3.77	N
	ATOM	698	C	HIS	92A	-33.036	74.677	-61.243	1.00	2.36	C
	ATOM	699	O	HIS	92A	-33.082	75.478	-60.301	1.00	1.78	O
	ATOM	700	N	ASP	93A	-32.373	73.522	-61.207	1.00	1.80	N
	ATOM	701	CA	ASP	93A	-31.610	73.102	-60.036	1.00	1.22	C
	ATOM	702	CB	ASP	93A	-31.261	71.604	-60.113	1.00	1.00	C
35	ATOM	703	CG	ASP	93A	-30.614	71.204	-61.438	1.00	2.03	C
	ATOM	704	OD1	ASP	93A	-31.238	71.393	-62.497	1.00	4.82	O
	ATOM	705	OD2	ASP	93A	-29.478	70.684	-61.423	1.00	2.22	O
	ATOM	706	C	ASP	93A	-30.340	73.939	-59.946	1.00	1.11	C
	ATOM	707	O	ASP	93A	-29.736	74.286	-60.966	1.00	1.00	O
40	ATOM	708	N	GLU	94A	-29.933	74.267	-58.725	1.00	1.89	N
	ATOM	709	CA	GLU	94A	-28.747	75.095	-58.540	1.00	1.00	C
	ATOM	710	CB	GLU	94A	-29.150	76.478	-58.006	1.00	1.00	C
	ATOM	711	CG	GLU	94A	-27.963	77.399	-57.699	1.00	1.00	C
	ATOM	712	CD	GLU	94A	-27.097	77.656	-58.912	1.00	4.34	C
45	ATOM	713	OE1	GLU	94A	-27.569	78.321	-59.859	1.00	2.87	O
	ATOM	714	OE2	GLU	94A	-25.937	77.185	-58.923	1.00	6.65	O
	ATOM	715	C	GLU	94A	-27.730	74.454	-57.614	1.00	1.02	C
	ATOM	716	O	GLU	94A	-27.988	74.263	-56.425	1.00	1.00	O
	ATOM	717	N	LEU	95A	-26.570	74.136	-58.175	1.00	1.00	N
50	ATOM	718	CA	LEU	95A	-25.494	73.509	-57.423	1.00	2.70	C
	ATOM	719	CB	LEU	95A	-24.397	73.028	-58.387	1.00	2.52	C
	ATOM	720	CG	LEU	95A	-24.745	71.889	-59.360	1.00	1.00	C
	ATOM	721	CD1	LEU	95A	-23.758	71.878	-60.527	1.00	1.99	C
	ATOM	722	CD2	LEU	95A	-24.729	70.555	-58.630	1.00	1.00	C
55	ATOM	723	C	LEU	95A	-24.878	74.432	-56.369	1.00	2.71	C
	ATOM	724	O	LEU	95A	-24.463	73.962	-55.306	1.00	2.36	O
	ATOM	725	N	ASP	96A	-24.838	75.735	-56.648	1.00	2.51	N
	ATOM	726	CA	ASP	96A	-24.220	76.692	-55.723	1.00	2.80	C
	ATOM	727	CB	ASP	96A	-23.729	77.921	-56.491	1.00	3.18	C
60	ATOM	728	CG	ASP	96A	-22.528	77.616	-57.364	1.00	5.63	C
	ATOM	729	OD1	ASP	96A	-21.889	76.560	-57.156	1.00	7.10	O

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	ATOM	730	OD2	ASP	96A	-22.213	78.433	-58.251	1.00	6.78	O
	ATOM	731	C	ASP	96A	-25.014	77.133	-54.498	1.00	3.41	C
	ATOM	732	O	ASP	96A	-24.562	77.994	-53.736	1.00	1.00	O
5	ATOM	733	N	MET	97A	-26.189	76.543	-54.311	1.00	1.00	N
	ATOM	734	CA	MET	97A	-27.033	76.829	-53.160	1.00	2.15	C
	ATOM	735	CB	MET	97A	-28.277	77.608	-53.586	1.00	2.06	C
	ATOM	736	CG	MET	97A	-27.976	78.995	-54.111	1.00	5.02	C
	ATOM	737	SD	MET	97A	-29.393	79.771	-54.919	1.00	11.41	S
10	ATOM	738	CE	MET	97A	-28.534	80.768	-56.133	1.00	7.74	C
	ATOM	739	C	MET	97A	-27.422	75.467	-52.592	1.00	3.33	C
	ATOM	740	O	MET	97A	-27.658	74.524	-53.344	1.00	3.24	O
	ATOM	741	N	PRO	98A	-27.479	75.338	-51.260	1.00	2.62	N
	ATOM	742	CD	PRO	98A	-27.055	76.285	-50.208	1.00	4.54	C
15	ATOM	743	CA	PRO	98A	-27.844	74.041	-50.686	1.00	3.39	C
	ATOM	744	CB	PRO	98A	-27.249	74.113	-49.285	1.00	1.00	C
	ATOM	745	CG	PRO	98A	-27.470	75.563	-48.927	1.00	1.91	C
	ATOM	746	C	PRO	98A	-29.348	73.792	-50.645	1.00	1.62	C
	ATOM	747	O	PRO	98A	-30.154	74.704	-50.864	1.00	3.59	O
20	ATOM	748	N	PRO	99A	-29.746	72.544	-50.368	1.00	5.30	N
	ATOM	749	CD	PRO	99A	-28.937	71.320	-50.230	1.00	2.20	C
	ATOM	750	CA	PRO	99A	-31.171	72.231	-50.299	1.00	4.15	C
	ATOM	751	CB	PRO	99A	-31.175	70.773	-49.852	1.00	4.44	C
	ATOM	752	CG	PRO	99A	-29.957	70.232	-50.508	1.00	5.09	C
25	ATOM	753	C	PRO	99A	-31.826	73.149	-49.267	1.00	6.14	C
	ATOM	754	O	PRO	99A	-31.299	73.323	-48.167	1.00	8.10	O
	ATOM	755	N	GLY	100A	-32.965	73.733	-49.624	1.00	5.74	N
	ATOM	756	CA	GLY	100A	-33.659	74.621	-48.709	1.00	5.37	C
	ATOM	757	C	GLY	100A	-33.565	76.076	-49.118	1.00	7.00	C
30	ATOM	758	O	GLY	100A	-34.282	76.928	-48.593	1.00	6.78	O
	ATOM	759	N	VAL	101A	-32.688	76.361	-50.073	1.00	6.83	N
	ATOM	760	CA	VAL	101A	-32.494	77.723	-50.549	1.00	8.14	C
	ATOM	761	CB	VAL	101A	-31.007	78.121	-50.455	1.00	7.01	C
	ATOM	762	CG1	VAL	101A	-30.789	79.503	-51.071	1.00	6.16	C
35	ATOM	763	CG2	VAL	101A	-30.571	78.115	-48.995	1.00	6.74	C
	ATOM	764	C	VAL	101A	-32.977	77.944	-51.982	1.00	8.68	C
	ATOM	765	O	VAL	101A	-32.639	77.183	-52.891	1.00	9.42	O
	ATOM	766	N	ALA	102A	-33.769	78.995	-52.170	1.00	6.76	N
	ATOM	767	CA	ALA	102A	-34.293	79.343	-53.483	1.00	6.36	C
40	ATOM	768	CB	ALA	102A	-35.790	79.058	-53.549	1.00	7.15	C
	ATOM	769	C	ALA	102A	-34.030	80.821	-53.750	1.00	6.69	C
	ATOM	770	O	ALA	102A	-34.325	81.671	-52.914	1.00	5.32	O
	ATOM	771	N	LYS	103A	-33.452	81.123	-54.906	1.00	5.55	N
	ATOM	772	CA	LYS	103A	-33.182	82.507	-55.252	1.00	5.58	C
45	ATOM	773	CB	LYS	103A	-31.718	82.854	-54.978	1.00	9.03	C
	ATOM	774	CG	LYS	103A	-31.359	82.756	-53.508	1.00	11.36	C
	ATOM	775	CD	LYS	103A	-30.098	83.539	-53.181	1.00	13.50	C
	ATOM	776	CE	LYS	103A	-29.613	83.226	-51.771	1.00	16.98	C
	ATOM	777	NZ	LYS	103A	-30.674	83.450	-50.745	1.00	17.15	N
50	ATOM	778	C	LYS	103A	-33.548	82.825	-56.694	1.00	5.53	C
	ATOM	779	O	LYS	103A	-33.606	81.937	-57.548	1.00	3.59	O
	ATOM	780	N	LEU	104A	-33.823	84.101	-56.947	1.00	3.31	N
	ATOM	781	CA	LEU	104A	-34.197	84.565	-58.273	1.00	3.87	C
	ATOM	782	CB	LEU	104A	-35.455	85.442	-58.190	1.00	3.81	C
55	ATOM	783	CG	LEU	104A	-36.766	84.741	-57.822	1.00	1.92	C
	ATOM	784	CD1	LEU	104A	-37.811	85.737	-57.343	1.00	5.97	C
	ATOM	785	CD2	LEU	104A	-37.265	83.977	-59.039	1.00	1.35	C
	ATOM	786	C	LEU	104A	-33.050	85.371	-58.872	1.00	4.91	C
	ATOM	787	O	LEU	104A	-32.307	86.045	-58.156	1.00	7.01	O
60	ATOM	788	N	LYS	105A	-32.896	85.285	-60.184	1.00	5.26	N
	ATOM	789	CA	LYS	105A	-31.843	86.024	-60.863	1.00	8.68	C
	ATOM	790	CB	LYS	105A	-30.539	85.222	-60.885	1.00	9.23	C

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	ATOM	791	CG	LYS	105A	-29.405	85.974	-61.565	1.00	14.33	C
	ATOM	792	CD	LYS	105A	-28.132	85.159	-61.638	1.00	18.70	C
	ATOM	793	CE	LYS	105A	-27.089	85.880	-62.481	1.00	19.07	C
	ATOM	794	NZ	LYS	105A	-25.876	85.052	-62.695	1.00	21.33	N
5	ATOM	795	C	LYS	105A	-32.264	86.337	-62.284	1.00	8.50	C
	ATOM	796	O	LYS	105A	-32.954	85.551	-62.923	1.00	7.17	O
	ATOM	797	N	THR	106A	-31.850	87.494	-62.776	1.00	9.20	N
	ATOM	798	CA	THR	106A	-32.187	87.881	-64.134	1.00	11.88	C
	ATOM	799	CB	THR	106A	-32.861	89.271	-64.162	1.00	13.82	C
10	ATOM	800	OG1	THR	106A	-33.007	89.710	-65.517	1.00	16.55	O
	ATOM	801	CG2	THR	106A	-32.036	90.275	-63.393	1.00	15.81	C
	ATOM	802	C	THR	106A	-30.913	87.892	-64.972	1.00	12.59	C
	ATOM	803	O	THR	106A	-29.881	88.418	-64.547	1.00	9.88	O
	ATOM	804	N	GLY	107A	-30.984	87.280	-66.148	1.00	12.58	N
15	ATOM	805	CA	GLY	107A	-29.832	87.234	-67.026	1.00	15.66	C
	ATOM	806	C	GLY	107A	-28.661	86.457	-66.461	1.00	16.09	C
	ATOM	807	O	GLY	107A	-28.804	85.671	-65.522	1.00	16.17	O
	ATOM	808	N	GLY	108A	-27.488	86.682	-67.038	1.00	18.04	N
	ATOM	809	CA	GLY	108A	-26.302	85.986	-66.583	1.00	17.36	C
20	ATOM	810	C	GLY	108A	-26.044	84.761	-67.438	1.00	18.87	C
	ATOM	811	O	GLY	108A	-26.798	84.479	-68.375	1.00	18.34	O
	ATOM	812	N	GLY	109A	-24.986	84.028	-67.107	1.00	18.26	N
	ATOM	813	CA	GLY	109A	-24.640	82.837	-67.862	1.00	17.43	C
	ATOM	814	C	GLY	109A	-25.479	81.620	-67.518	1.00	16.37	C
25	ATOM	815	O	GLY	109A	-26.601	81.738	-67.028	1.00	16.42	O
	ATOM	816	N	HIS	110A	-24.928	80.441	-67.775	1.00	15.39	N
	ATOM	817	CA	HIS	110A	-25.626	79.193	-67.500	1.00	15.16	C
	ATOM	818	CB	HIS	110A	-25.635	78.318	-68.754	1.00	14.88	C
	ATOM	819	CG	HIS	110A	-24.282	78.129	-69.362	1.00	15.15	C
30	ATOM	820	CD2	HIS	110A	-23.803	78.442	-70.589	1.00	14.18	C
	ATOM	821	ND1	HIS	110A	-23.234	77.555	-68.677	1.00	14.24	N
	ATOM	822	CE1	HIS	110A	-22.167	77.524	-69.456	1.00	15.94	C
	ATOM	823	NE2	HIS	110A	-22.486	78.056	-70.621	1.00	13.76	N
	ATOM	824	C	HIS	110A	-24.989	78.429	-66.345	1.00	15.27	C
35	ATOM	825	O	HIS	110A	-25.562	77.466	-65.841	1.00	13.41	O
	ATOM	826	N	GLY	111A	-23.803	78.865	-65.927	1.00	16.21	N
	ATOM	827	CA	GLY	111A	-23.113	78.207	-64.830	1.00	15.78	C
	ATOM	828	C	GLY	111A	-22.869	76.726	-65.053	1.00	16.47	C
	ATOM	829	O	GLY	111A	-22.812	75.950	-64.100	1.00	16.61	O
40	ATOM	830	N	GLY	112A	-22.717	76.329	-66.314	1.00	15.55	N
	ATOM	831	CA	GLY	112A	-22.483	74.931	-66.623	1.00	15.17	C
	ATOM	832	C	GLY	112A	-23.753	74.107	-66.755	1.00	13.15	C
	ATOM	833	O	GLY	112A	-23.702	72.948	-67.166	1.00	16.31	O
	ATOM	834	N	HIS	113A	-24.896	74.692	-66.409	1.00	12.94	N
45	ATOM	835	CA	HIS	113A	-26.158	73.973	-66.512	1.00	10.81	C
	ATOM	836	CB	HIS	113A	-27.266	74.722	-65.770	1.00	8.88	C
	ATOM	837	CG	HIS	113A	-28.505	73.907	-65.571	1.00	6.89	C
	ATOM	838	CD2	HIS	113A	-29.075	73.409	-64.448	1.00	5.22	C
	ATOM	839	ND1	HIS	113A	-29.289	73.480	-66.620	1.00	8.12	N
50	ATOM	840	CE1	HIS	113A	-30.287	72.752	-66.154	1.00	5.50	C
	ATOM	841	NE2	HIS	113A	-30.181	72.692	-64.837	1.00	5.53	N
	ATOM	842	C	HIS	113A	-26.491	73.858	-67.995	1.00	9.87	C
	ATOM	843	O	HIS	113A	-26.792	74.850	-68.656	1.00	10.05	O
	ATOM	844	N	ASN	114A	-26.422	72.638	-68.512	1.00	10.16	N
55	ATOM	845	CA	ASN	114A	-26.667	72.392	-69.923	1.00	10.50	C
	ATOM	846	CB	ASN	114A	-26.256	70.959	-70.251	1.00	14.98	C
	ATOM	847	CG	ASN	114A	-24.774	70.736	-70.026	1.00	18.32	C
	ATOM	848	OD1	ASN	114A	-24.314	69.608	-69.857	1.00	20.80	O
	ATOM	849	ND2	ASN	114A	-24.014	71.828	-70.024	1.00	19.91	N
60	ATOM	850	C	ASN	114A	-28.078	72.692	-70.397	1.00	9.08	C
	ATOM	851	O	ASN	114A	-28.305	72.873	-71.593	1.00	6.27	O

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	ATOM	852	N	GLY	115A	-29.021	72.755	-69.461	1.00	7.09	N
	ATOM	853	CA	GLY	115A	-30.384	73.081	-69.833	1.00	6.38	C
	ATOM	854	C	GLY	115A	-30.415	74.569	-70.121	1.00	4.56	C
	ATOM	855	O	GLY	115A	-30.936	75.016	-71.147	1.00	3.76	O
5	ATOM	856	N	LEU	116A	-29.842	75.345	-69.207	1.00	5.41	N
	ATOM	857	CA	LEU	116A	-29.785	76.789	-69.362	1.00	5.93	C
	ATOM	858	CB	LEU	116A	-29.159	77.444	-68.122	1.00	7.99	C
	ATOM	859	CG	LEU	116A	-29.963	77.454	-66.819	1.00	11.76	C
	ATOM	860	CD1	LEU	116A	-29.132	78.075	-65.701	1.00	12.07	C
	ATOM	861	CD2	LEU	116A	-31.254	78.249	-67.025	1.00	11.88	C
10	ATOM	862	C	LEU	116A	-28.946	77.119	-70.593	1.00	5.55	C
	ATOM	863	O	LEU	116A	-29.282	78.014	-71.360	1.00	4.53	O
	ATOM	864	N	LYS	117A	-27.859	76.377	-70.785	1.00	5.46	N
	ATOM	865	CA	LYS	117A	-26.984	76.609	-71.928	1.00	6.12	C
	ATOM	866	CB	LYS	117A	-25.824	75.601	-71.927	1.00	5.51	C
	ATOM	867	CG	LYS	117A	-24.880	75.759	-73.114	1.00	8.75	C
15	ATOM	868	CD	LYS	117A	-23.552	75.025	-72.915	1.00	4.61	C
	ATOM	869	CE	LYS	117A	-23.698	73.527	-72.753	1.00	9.26	C
	ATOM	870	NZ	LYS	117A	-22.348	72.894	-72.555	1.00	11.81	N
	ATOM	871	C	LYS	117A	-27.735	76.530	-73.261	1.00	6.70	C
	ATOM	872	O	LYS	117A	-27.581	77.405	-74.117	1.00	7.74	O
	ATOM	873	N	ASP	118A	-28.554	75.495	-73.426	1.00	6.22	N
20	ATOM	874	CA	ASP	118A	-29.304	75.304	-74.665	1.00	5.45	C
	ATOM	875	CB	ASP	118A	-29.932	73.902	-74.693	1.00	2.99	C
	ATOM	876	CG	ASP	118A	-30.352	73.481	-76.092	1.00	3.70	C
	ATOM	877	OD1	ASP	118A	-29.478	73.426	-76.982	1.00	2.25	O
	ATOM	878	OD2	ASP	118A	-31.549	73.206	-76.312	1.00	1.28	O
	ATOM	879	C	ASP	118A	-30.387	76.366	-74.836	1.00	4.76	C
25	ATOM	880	O	ASP	118A	-30.643	76.841	-75.943	1.00	5.67	O
	ATOM	881	N	ILE	119A	-31.035	76.732	-73.739	1.00	4.75	N
	ATOM	882	CA	ILE	119A	-32.070	77.745	-73.807	1.00	4.40	C
	ATOM	883	CB	ILE	119A	-32.754	77.936	-72.441	1.00	2.72	C
	ATOM	884	CG2	ILE	119A	-33.725	79.105	-72.506	1.00	2.29	C
	ATOM	885	CG1	ILE	119A	-33.510	76.653	-72.069	1.00	2.63	C
30	ATOM	886	CD1	ILE	119A	-34.062	76.649	-70.655	1.00	2.60	C
	ATOM	887	C	ILE	119A	-31.454	79.059	-74.272	1.00	5.25	C
	ATOM	888	O	ILE	119A	-31.993	79.725	-75.156	1.00	5.20	O
	ATOM	889	N	ILE	120A	-30.323	79.428	-73.682	1.00	6.96	N
	ATOM	890	CA	ILE	120A	-29.660	80.665	-74.078	1.00	9.05	C
	ATOM	891	CB	ILE	120A	-28.400	80.937	-73.238	1.00	9.93	C
35	ATOM	892	CG2	ILE	120A	-27.643	82.129	-73.810	1.00	12.52	C
	ATOM	893	CG1	ILE	120A	-28.789	81.217	-71.787	1.00	12.36	C
	ATOM	894	CD1	ILE	120A	-27.605	81.385	-70.862	1.00	12.03	C
	ATOM	895	C	ILE	120A	-29.257	80.589	-75.546	1.00	9.38	C
	ATOM	896	O	ILE	120A	-29.477	81.531	-76.301	1.00	12.07	O
	ATOM	897	N	ALA	121A	-28.676	79.464	-75.950	1.00	9.92	N
40	ATOM	898	CA	ALA	121A	-28.240	79.293	-77.334	1.00	9.79	C
	ATOM	899	CB	ALA	121A	-27.520	77.959	-77.501	1.00	9.99	C
	ATOM	900	C	ALA	121A	-29.415	79.372	-78.298	1.00	12.25	C
	ATOM	901	O	ALA	121A	-29.327	79.995	-79.356	1.00	13.32	O
	ATOM	902	N	GLN	122A	-30.522	78.746	-77.922	1.00	12.36	N
	ATOM	903	CA	GLN	122A	-31.700	78.734	-78.770	1.00	17.39	C
45	ATOM	904	CB	GLN	122A	-32.719	77.735	-78.224	1.00	20.81	C
	ATOM	905	CG	GLN	122A	-33.650	77.198	-79.283	1.00	26.52	C
	ATOM	906	CD	GLN	122A	-32.900	76.490	-80.388	1.00	28.82	C
	ATOM	907	OE1	GLN	122A	-32.330	75.417	-80.181	1.00	31.07	O
	ATOM	908	NE2	GLN	122A	-32.884	77.094	-81.571	1.00	31.63	N
	ATOM	909	C	GLN	122A	-32.348	80.107	-78.916	1.00	18.24	C
50	ATOM	910	O	GLN	122A	-32.781	80.480	-80.004	1.00	18.95	O
	ATOM	911	N	LEU	123A	-32.416	80.865	-77.827	1.00	17.48	N
	ATOM	912	CA	LEU	123A	-33.036	82.181	-77.893	1.00	20.57	C

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	ATOM	913	CB	LEU	123A	-33.470	82.635	-76.494	1.00	15.46	C
	ATOM	914	CG	LEU	123A	-34.530	81.734	-75.841	1.00	15.80	C
	ATOM	915	CD1	LEU	123A	-34.938	82.302	-74.494	1.00	12.14	C
	ATOM	916	CD2	LEU	123A	-35.740	81.609	-76.759	1.00	14.23	C
5	ATOM	917	C	LEU	123A	-32.096	83.200	-78.532	1.00	24.33	C
	ATOM	918	O	LEU	123A	-32.019	84.352	-78.102	1.00	24.22	O
	ATOM	919	N	GLY	124A	-31.393	82.760	-79.573	1.00	27.34	N
	ATOM	920	CA	GLY	124A	-30.464	83.625	-80.278	1.00	31.47	C
	ATOM	921	C	GLY	124A	-29.503	84.337	-79.349	1.00	33.87	C
	ATOM	922	O	GLY	124A	-29.284	85.542	-79.479	1.00	36.58	O
10	ATOM	923	N	ASN	125A	-28.929	83.591	-78.410	1.00	35.87	N
	ATOM	924	CA	ASN	125A	-27.989	84.147	-77.443	1.00	35.97	C
	ATOM	925	CB	ASN	125A	-26.801	84.796	-78.163	1.00	38.93	C
	ATOM	926	CG	ASN	125A	-25.827	83.774	-78.730	1.00	40.22	C
	ATOM	927	OD1	ASN	125A	-24.886	84.128	-79.443	1.00	41.68	O
15	ATOM	928	ND2	ASN	125A	-26.043	82.503	-78.407	1.00	40.73	N
	ATOM	929	C	ASN	125A	-28.650	85.167	-76.520	1.00	34.54	C
	ATOM	930	O	ASN	125A	-28.031	86.160	-76.135	1.00	35.18	O
	ATOM	931	N	ASN	126A	-29.909	84.921	-76.170	1.00	33.49	N
	ATOM	932	CA	ASN	126A	-30.638	85.817	-75.280	1.00	33.84	C
20	ATOM	933	CB	ASN	126A	-32.002	86.183	-75.872	1.00	32.37	C
	ATOM	934	CG	ASN	126A	-31.907	87.262	-76.933	1.00	31.11	C
	ATOM	935	OD1	ASN	126A	-32.918	87.704	-77.476	1.00	30.83	O
	ATOM	936	ND2	ASN	126A	-30.686	87.694	-77.231	1.00	32.10	N
	ATOM	937	C	ASN	126A	-30.831	85.210	-73.898	1.00	34.67	C
25	ATOM	938	O	ASN	126A	-31.801	84.496	-73.650	1.00	33.44	O
	ATOM	939	N	ASN	127A	-29.897	85.502	-73.000	1.00	34.58	N
	ATOM	940	CA	ASN	127A	-29.962	84.995	-71.635	1.00	34.82	C
	ATOM	941	CB	ASN	127A	-28.550	84.797	-71.081	1.00	39.11	C
	ATOM	942	CG	ASN	127A	-27.732	86.074	-71.103	1.00	42.27	C
30	ATOM	943	OD1	ASN	127A	-27.482	86.646	-72.164	1.00	43.93	O
	ATOM	944	ND2	ASN	127A	-27.311	86.528	-69.927	1.00	45.03	N
	ATOM	945	C	ASN	127A	-30.723	85.992	-70.769	1.00	31.48	C
	ATOM	946	O	ASN	127A	-30.757	85.872	-69.543	1.00	32.55	O
	ATOM	947	N	SER	128A	-31.339	86.971	-71.426	1.00	27.79	N
35	ATOM	948	CA	SER	128A	-32.094	88.014	-70.745	1.00	22.25	C
	ATOM	949	CB	SER	128A	-32.316	89.194	-71.696	1.00	23.86	C
	ATOM	950	OG	SER	128A	-32.902	88.768	-72.914	1.00	27.87	O
	ATOM	951	C	SER	128A	-33.433	87.545	-70.167	1.00	17.21	C
	ATOM	952	O	SER	128A	-34.367	88.334	-70.032	1.00	16.09	O
40	ATOM	953	N	PHE	129A	-33.525	86.263	-69.828	1.00	11.41	N
	ATOM	954	CA	PHE	129A	-34.744	85.729	-69.229	1.00	9.15	C
	ATOM	955	CB	PHE	129A	-35.115	84.383	-69.857	1.00	5.10	C
	ATOM	956	CG	PHE	129A	-33.998	83.385	-69.854	1.00	3.64	C
	ATOM	957	CD1	PHE	129A	-33.822	82.517	-68.781	1.00	3.05	C
45	ATOM	958	CD2	PHE	129A	-33.124	83.306	-70.932	1.00	3.02	C
	ATOM	959	CE1	PHE	129A	-32.792	81.578	-68.785	1.00	6.64	C
	ATOM	960	CE2	PHE	129A	-32.089	82.372	-70.948	1.00	4.68	C
	ATOM	961	CZ	PHE	129A	-31.923	81.507	-69.875	1.00	6.52	C
	ATOM	962	C	PHE	129A	-34.471	85.568	-67.739	1.00	5.65	C
50	ATOM	963	O	PHE	129A	-33.322	85.598	-67.317	1.00	6.79	O
	ATOM	964	N	HIS	130A	-35.521	85.393	-66.943	1.00	5.39	N
	ATOM	965	CA	HIS	130A	-35.352	85.262	-65.505	1.00	3.48	C
	ATOM	966	CB	HIS	130A	-36.510	85.959	-64.796	1.00	3.77	C
	ATOM	967	CG	HIS	130A	-36.729	87.359	-65.274	1.00	6.65	C
55	ATOM	968	CD2	HIS	130A	-36.193	88.531	-64.864	1.00	6.30	C
	ATOM	969	ND1	HIS	130A	-37.525	87.659	-66.358	1.00	8.43	N
	ATOM	970	CE1	HIS	130A	-37.469	88.957	-66.597	1.00	5.55	C
	ATOM	971	NE2	HIS	130A	-36.667	89.509	-65.706	1.00	9.03	N
	ATOM	972	C	HIS	130A	-35.231	83.814	-65.063	1.00	3.06	C
60	ATOM	973	O	HIS	130A	-35.746	82.909	-65.722	1.00	1.63	O

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	ATOM	974	N	ARG	131A	-34.537	83.596	-63.951	1.00	3.89	N
	ATOM	975	CA	ARG	131A	-34.340	82.247	-63.448	1.00	3.99	C
	ATOM	976	CB	ARG	131A	-32.882	81.808	-63.650	1.00	5.92	C
	ATOM	977	CG	ARG	131A	-32.337	81.973	-65.064	1.00	7.19	C
5	ATOM	978	CD	ARG	131A	-31.906	83.401	-65.336	1.00	10.69	C
	ATOM	979	NE	ARG	131A	-31.195	83.549	-66.606	1.00	10.32	N
	ATOM	980	CZ	ARG	131A	-29.961	83.110	-66.842	1.00	12.78	C
	ATOM	981	NH1	ARG	131A	-29.277	82.480	-65.895	1.00	10.23	N
	ATOM	982	NH2	ARG	131A	-29.394	83.326	-68.025	1.00	13.36	N
	ATOM	983	C	ARG	131A	-34.691	82.067	-61.975	1.00	4.09	C
10	ATOM	984	O	ARG	131A	-34.407	82.933	-61.144	1.00	3.82	O
	ATOM	985	N	LEU	132A	-35.318	80.935	-61.667	1.00	3.73	N
	ATOM	986	CA	LEU	132A	-35.665	80.591	-60.296	1.00	3.43	C
	ATOM	987	CB	LEU	132A	-37.093	80.027	-60.216	1.00	5.42	C
	ATOM	988	CG	LEU	132A	-37.792	80.073	-58.848	1.00	9.25	C
15	ATOM	989	CD1	LEU	132A	-39.131	79.330	-58.902	1.00	8.43	C
	ATOM	990	CD2	LEU	132A	-36.917	79.449	-57.805	1.00	10.45	C
	ATOM	991	C	LEU	132A	-34.635	79.504	-59.973	1.00	3.69	C
	ATOM	992	O	LEU	132A	-34.710	78.389	-60.494	1.00	1.00	O
20	ATOM	993	N	ARG	133A	-33.657	79.841	-59.141	1.00	1.94	N
	ATOM	994	CA	ARG	133A	-32.596	78.902	-58.788	1.00	2.71	C
	ATOM	995	CB	ARG	133A	-31.289	79.664	-58.572	1.00	3.22	C
	ATOM	996	CG	ARG	133A	-30.960	80.589	-59.729	1.00	5.14	C
	ATOM	997	CD	ARG	133A	-29.811	81.513	-59.404	1.00	7.26	C
25	ATOM	998	NE	ARG	133A	-28.545	80.802	-59.292	1.00	7.21	N
	ATOM	999	CZ	ARG	133A	-27.380	81.397	-59.075	1.00	10.51	C
	ATOM	1000	NH1	ARG	133A	-27.329	82.715	-58.949	1.00	10.82	N
	ATOM	1001	NH2	ARG	133A	-26.266	80.678	-58.983	1.00	12.00	N
30	ATOM	1002	C	ARG	133A	-32.944	78.104	-57.545	1.00	4.29	C
	ATOM	1003	O	ARG	133A	-33.007	78.653	-56.448	1.00	3.68	O
	ATOM	1004	N	LEU	134A	-33.154	76.805	-57.739	1.00	1.00	N
	ATOM	1005	CA	LEU	134A	-33.516	75.891	-56.662	1.00	2.72	C
	ATOM	1006	CB	LEU	134A	-34.602	74.934	-57.155	1.00	4.09	C
35	ATOM	1007	CG	LEU	134A	-35.825	75.658	-57.726	1.00	4.01	C
	ATOM	1008	CD1	LEU	134A	-36.759	74.644	-58.370	1.00	1.00	C
	ATOM	1009	CD2	LEU	134A	-36.537	76.430	-56.615	1.00	6.05	C
	ATOM	1010	C	LEU	134A	-32.308	75.106	-56.167	1.00	2.50	C
	ATOM	1011	O	LEU	134A	-31.837	74.181	-56.832	1.00	2.89	O
40	ATOM	1012	N	GLY	135A	-31.818	75.477	-54.990	1.00	3.27	N
	ATOM	1013	CA	GLY	135A	-30.653	74.810	-54.430	1.00	2.97	C
	ATOM	1014	C	GLY	135A	-30.758	73.303	-54.287	1.00	1.55	C
	ATOM	1015	O	GLY	135A	-31.782	72.779	-53.853	1.00	2.43	O
	ATOM	1016	N	ILE	136A	-29.688	72.600	-54.652	1.00	1.80	N
45	ATOM	1017	CA	ILE	136A	-29.647	71.144	-54.546	1.00	2.11	C
	ATOM	1018	CB	ILE	136A	-29.766	70.457	-55.924	1.00	3.61	C
	ATOM	1019	CG2	ILE	136A	-31.155	70.681	-56.503	1.00	3.23	C
	ATOM	1020	CG1	ILE	136A	-28.692	70.992	-56.874	1.00	1.00	C
	ATOM	1021	CD1	ILE	136A	-28.394	70.046	-58.019	1.00	1.30	C
50	ATOM	1022	C	ILE	136A	-28.317	70.714	-53.933	1.00	1.47	C
	ATOM	1023	O	ILE	136A	-28.098	69.539	-53.655	1.00	1.00	O
	ATOM	1024	N	GLY	137A	-27.430	71.680	-53.722	1.00	2.06	N
	ATOM	1025	CA	GLY	137A	-26.127	71.369	-53.158	1.00	1.97	C
	ATOM	1026	C	GLY	137A	-25.098	71.000	-54.218	1.00	2.24	C
55	ATOM	1027	O	GLY	137A	-25.441	70.800	-55.384	1.00	1.00	O
	ATOM	1028	N	HIS	138A	-23.833	70.931	-53.809	1.00	1.38	N
	ATOM	1029	CA	HIS	138A	-22.733	70.581	-54.709	1.00	2.60	C
	ATOM	1030	CB	HIS	138A	-21.778	71.766	-54.867	1.00	3.56	C
	ATOM	1031	CG	HIS	138A	-20.804	71.607	-55.993	1.00	5.13	C
60	ATOM	1032	CD2	HIS	138A	-19.728	70.796	-56.131	1.00	4.96	C
	ATOM	1033	ND1	HIS	138A	-20.910	72.306	-57.177	1.00	6.28	N
	ATOM	1034	CE1	HIS	138A	-19.942	71.932	-57.995	1.00	4.76	C

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	ATOM	1035	NE2	HIS	138A	-19.212	71.015	-57.384	1.00	7.39	N
	ATOM	1036	C	HIS	138A	-21.976	69.383	-54.113	1.00	3.77	C
	ATOM	1037	O	HIS	138A	-21.717	69.347	-52.912	1.00	5.10	O
	ATOM	1038	N	PRO	139A	-21.614	68.387	-54.945	1.00	2.94	N
5	ATOM	1039	CD	PRO	139A	-21.846	68.295	-56.399	1.00	3.47	C
	ATOM	1040	CA	PRO	139A	-20.894	67.202	-54.465	1.00	3.90	C
	ATOM	1041	CB	PRO	139A	-20.976	66.250	-55.654	1.00	2.60	C
	ATOM	1042	CG	PRO	139A	-20.880	67.188	-56.811	1.00	2.19	C
	ATOM	1043	C	PRO	139A	-19.452	67.473	-54.056	1.00	5.14	C
10	ATOM	1044	O	PRO	139A	-18.816	66.632	-53.428	1.00	4.10	O
	ATOM	1045	N	GLY	140A	-18.945	68.643	-54.428	1.00	6.91	N
	ATOM	1046	CA	GLY	140A	-17.583	69.013	-54.102	1.00	11.14	C
	ATOM	1047	C	GLY	140A	-16.783	69.298	-55.361	1.00	14.48	C
	ATOM	1048	O	GLY	140A	-16.739	70.434	-55.843	1.00	16.27	O
15	ATOM	1049	N	HIS	141A	-16.160	68.264	-55.913	1.00	13.75	N
	ATOM	1050	CA	HIS	141A	-15.356	68.426	-57.116	1.00	13.95	C
	ATOM	1051	CB	HIS	141A	-14.313	67.313	-57.209	1.00	15.58	C
	ATOM	1052	CG	HIS	141A	-13.281	67.552	-58.266	1.00	17.24	C
	ATOM	1053	CD2	HIS	141A	-13.082	66.962	-59.467	1.00	16.54	C
20	ATOM	1054	ND1	HIS	141A	-12.320	68.535	-58.158	1.00	17.49	N
	ATOM	1055	CE1	HIS	141A	-11.575	68.539	-59.248	1.00	16.38	C
	ATOM	1056	NE2	HIS	141A	-12.017	67.595	-60.059	1.00	18.18	N
	ATOM	1057	C	HIS	141A	-16.219	68.421	-58.374	1.00	13.82	C
	ATOM	1058	O	HIS	141A	-17.232	67.723	-58.441	1.00	14.66	O
25	ATOM	1059	N	SER	142A	-15.805	69.200	-59.370	1.00	12.96	N
	ATOM	1060	CA	SER	142A	-16.534	69.310	-60.631	1.00	11.02	C
	ATOM	1061	CB	SER	142A	-15.802	70.268	-61.579	1.00	9.93	C
	ATOM	1062	OG	SER	142A	-14.481	69.823	-61.849	1.00	9.22	O
	ATOM	1063	C	SER	142A	-16.756	67.975	-61.333	1.00	12.17	C
30	ATOM	1064	O	SER	142A	-17.763	67.790	-62.022	1.00	9.42	O
	ATOM	1065	N	SER	143A	-15.823	67.045	-61.155	1.00	11.55	N
	ATOM	1066	CA	SER	143A	-15.926	65.735	-61.793	1.00	11.74	C
	ATOM	1067	CB	SER	143A	-14.622	64.948	-61.609	1.00	12.13	C
	ATOM	1068	OG	SER	143A	-14.411	64.622	-60.246	1.00	12.40	O
35	ATOM	1069	C	SER	143A	-17.089	64.886	-61.284	1.00	11.16	C
	ATOM	1070	O	SER	143A	-17.521	63.954	-61.967	1.00	10.69	O
	ATOM	1071	N	LEU	144A	-17.591	65.203	-60.091	1.00	9.26	N
	ATOM	1072	CA	LEU	144A	-18.691	64.443	-59.498	1.00	7.48	C
	ATOM	1073	CB	LEU	144A	-18.505	64.343	-57.983	1.00	7.45	C
40	ATOM	1074	CG	LEU	144A	-17.321	63.550	-57.421	1.00	8.05	C
	ATOM	1075	CD1	LEU	144A	-17.299	63.714	-55.905	1.00	9.06	C
	ATOM	1076	CD2	LEU	144A	-17.456	62.075	-57.787	1.00	8.30	C
	ATOM	1077	C	LEU	144A	-20.077	65.023	-59.774	1.00	7.06	C
	ATOM	1078	O	LEU	144A	-21.090	64.434	-59.388	1.00	4.53	O
45	ATOM	1079	N	VAL	145A	-20.119	66.163	-60.449	1.00	6.61	N
	ATOM	1080	CA	VAL	145A	-21.378	66.830	-60.739	1.00	7.34	C
	ATOM	1081	CB	VAL	145A	-21.124	68.174	-61.464	1.00	7.28	C
	ATOM	1082	CG1	VAL	145A	-22.442	68.853	-61.792	1.00	8.03	C
	ATOM	1083	CG2	VAL	145A	-20.278	69.084	-60.580	1.00	10.51	C
50	ATOM	1084	C	VAL	145A	-22.394	66.005	-61.532	1.00	8.25	C
	ATOM	1085	O	VAL	145A	-23.554	65.916	-61.134	1.00	7.56	O
	ATOM	1086	N	SER	146A	-21.977	65.406	-62.643	1.00	6.30	N
	ATOM	1087	CA	SER	146A	-22.898	64.607	-63.456	1.00	9.28	C
	ATOM	1088	CB	SER	146A	-22.155	63.942	-64.619	1.00	13.00	C
55	ATOM	1089	OG	SER	146A	-21.782	64.894	-65.600	1.00	14.89	O
	ATOM	1090	C	SER	146A	-23.643	63.536	-62.664	1.00	8.27	C
	ATOM	1091	O	SER	146A	-24.873	63.477	-62.701	1.00	9.47	O
	ATOM	1092	N	GLY	147A	-22.906	62.688	-61.955	1.00	5.67	N
	ATOM	1093	CA	GLY	147A	-23.545	61.641	-61.181	1.00	5.64	C
60	ATOM	1094	C	GLY	147A	-24.359	62.185	-60.021	1.00	5.45	C
	ATOM	1095	O	GLY	147A	-25.291	61.545	-59.540	1.00	4.99	O

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	ATOM	1096	N	TYR	148A	-24.009	63.378	-59.566	1.00	4.05	N
	ATOM	1097	CA	TYR	148A	-24.712	63.989	-58.448	1.00	4.39	C
	ATOM	1098	CB	TYR	148A	-23.875	65.136	-57.888	1.00	3.04	C
	ATOM	1099	CG	TYR	148A	-24.486	65.820	-56.685	1.00	4.05	C
5	ATOM	1100	CD1	TYR	148A	-24.334	65.290	-55.401	1.00	2.82	C
	ATOM	1101	CE1	TYR	148A	-24.871	65.934	-54.293	1.00	3.66	C
	ATOM	1102	CD2	TYR	148A	-25.196	67.010	-56.830	1.00	1.00	C
	ATOM	1103	CE2	TYR	148A	-25.737	67.662	-55.733	1.00	2.06	C
	ATOM	1104	CZ	TYR	148A	-25.571	67.121	-54.470	1.00	3.84	C
	ATOM	1105	OH	TYR	148A	-26.101	67.771	-53.390	1.00	2.60	O
10	ATOM	1106	C	TYR	148A	-26.098	64.507	-58.829	1.00	4.46	C
	ATOM	1107	O	TYR	148A	-27.084	64.218	-58.147	1.00	4.47	O
	ATOM	1108	N	VAL	149A	-26.177	65.269	-59.915	1.00	3.43	N
	ATOM	1109	CA	VAL	149A	-27.460	65.827	-60.329	1.00	1.00	C
	ATOM	1110	CB	VAL	149A	-27.291	66.990	-61.342	1.00	2.54	C
	ATOM	1111	CG1	VAL	149A	-26.455	68.098	-60.718	1.00	2.54	C
15	ATOM	1112	CG2	VAL	149A	-26.642	66.485	-62.625	1.00	3.20	C
	ATOM	1113	C	VAL	149A	-28.355	64.767	-60.938	1.00	2.51	C
	ATOM	1114	O	VAL	149A	-29.562	64.948	-61.023	1.00	1.00	O
	ATOM	1115	N	LEU	150A	-27.763	63.655	-61.351	1.00	1.00	N
	ATOM	1116	CA	LEU	150A	-28.547	62.579	-61.941	1.00	3.02	C
	ATOM	1117	CB	LEU	150A	-27.760	61.902	-63.062	1.00	3.49	C
20	ATOM	1118	CG	LEU	150A	-27.362	62.841	-64.206	1.00	5.59	C
	ATOM	1119	CD1	LEU	150A	-26.635	62.051	-65.276	1.00	6.87	C
	ATOM	1120	CD2	LEU	150A	-28.597	63.535	-64.777	1.00	4.33	C
	ATOM	1121	C	LEU	150A	-28.928	61.566	-60.874	1.00	3.21	C
	ATOM	1122	O	LEU	150A	-29.521	60.530	-61.167	1.00	1.39	O
	ATOM	1123	N	GLY	151A	-28.582	61.871	-59.628	1.00	3.02	N
25	ATOM	1124	CA	GLY	151A	-28.910	60.966	-58.545	1.00	5.57	C
	ATOM	1125	C	GLY	151A	-30.136	61.411	-57.777	1.00	6.11	C
	ATOM	1126	O	GLY	151A	-30.537	62.568	-57.852	1.00	3.26	O
	ATOM	1127	N	ARG	152A	-30.752	60.487	-57.052	1.00	7.64	N
	ATOM	1128	CA	ARG	152A	-31.919	60.827	-56.256	1.00	9.22	C
	ATOM	1129	CB	ARG	152A	-32.901	59.654	-56.208	1.00	13.25	C
30	ATOM	1130	CG	ARG	152A	-33.505	59.338	-57.567	1.00	17.92	C
	ATOM	1131	CD	ARG	152A	-34.493	58.183	-57.505	1.00	22.67	C
	ATOM	1132	NE	ARG	152A	-34.878	57.717	-58.837	1.00	27.01	N
	ATOM	1133	CZ	ARG	152A	-35.584	58.426	-59.714	1.00	28.52	C
	ATOM	1134	NH1	ARG	152A	-35.995	59.648	-59.411	1.00	28.94	N
	ATOM	1135	NH2	ARG	152A	-35.888	57.911	-60.897	1.00	28.72	N
35	ATOM	1136	C	ARG	152A	-31.421	61.183	-54.867	1.00	7.91	C
	ATOM	1137	O	ARG	152A	-30.754	60.391	-54.206	1.00	9.42	O
	ATOM	1138	N	ALA	153A	-31.738	62.395	-54.433	1.00	7.52	N
	ATOM	1139	CA	ALA	153A	-31.289	62.868	-53.136	1.00	9.09	C
	ATOM	1140	CB	ALA	153A	-31.548	64.363	-53.024	1.00	8.87	C
	ATOM	1141	C	ALA	153A	-31.956	62.147	-51.975	1.00	8.66	C
40	ATOM	1142	O	ALA	153A	-33.076	61.646	-52.102	1.00	9.81	O
	ATOM	1143	N	PRO	154A	-31.259	62.063	-50.828	1.00	10.19	N
	ATOM	1144	CD	PRO	154A	-29.884	62.512	-50.555	1.00	10.91	C
	ATOM	1145	CA	PRO	154A	-31.839	61.398	-49.657	1.00	10.87	C
	ATOM	1146	CB	PRO	154A	-30.704	61.445	-48.629	1.00	10.36	C
	ATOM	1147	CG	PRO	154A	-29.882	62.622	-49.053	1.00	12.30	C
45	ATOM	1148	C	PRO	154A	-33.060	62.226	-49.255	1.00	10.57	C
	ATOM	1149	O	PRO	154A	-33.146	63.404	-49.608	1.00	7.67	O
	ATOM	1150	N	ARG	155A	-34.000	61.633	-48.528	1.00	11.24	N
	ATOM	1151	CA	ARG	155A	-35.212	62.358	-48.161	1.00	15.17	C
	ATOM	1152	CB	ARG	155A	-36.145	61.470	-47.340	1.00	19.61	C
	ATOM	1153	CG	ARG	155A	-35.729	61.300	-45.899	1.00	26.57	C
50	ATOM	1154	CD	ARG	155A	-36.931	60.958	-45.052	1.00	32.23	C
	ATOM	1155	NE	ARG	155A	-36.618	60.967	-43.629	1.00	37.23	N
55	ATOM	1156	CZ	ARG	155A	-36.168	62.030	-42.968	1.00	39.78	C

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	ATOM	1157	NH1	ARG	155A	-35.970	63.183	-43.600	1.00	39.27	N
	ATOM	1158	NH2	ARG	155A	-35.920	61.941	-41.668	1.00	41.07	N
	ATOM	1159	C	ARG	155A	-34.996	63.677	-47.422	1.00	11.94	C
	ATOM	1160	O	ARG	155A	-35.750	64.625	-47.623	1.00	11.79	O
5	ATOM	1161	N	SER	156A	-33.978	63.749	-46.572	1.00	11.69	N
	ATOM	1162	CA	SER	156A	-33.726	64.984	-45.835	1.00	10.21	C
	ATOM	1163	CB	SER	156A	-32.522	64.821	-44.903	1.00	13.03	C
	ATOM	1164	OG	SER	156A	-31.327	64.615	-45.634	1.00	14.20	O
	ATOM	1165	C	SER	156A	-33.483	66.153	-46.789	1.00	9.66	C
10	ATOM	1166	O	SER	156A	-33.881	67.283	-46.512	1.00	8.09	O
	ATOM	1167	N	GLU	157A	-32.833	65.876	-47.915	1.00	7.66	N
	ATOM	1168	CA	GLU	157A	-32.550	66.910	-48.904	1.00	6.24	C
	ATOM	1169	CB	GLU	157A	-31.357	66.496	-49.767	1.00	4.69	C
	ATOM	1170	CG	GLU	157A	-30.028	66.524	-49.030	1.00	6.65	C
15	ATOM	1171	CD	GLU	157A	-28.879	66.013	-49.881	1.00	9.21	C
	ATOM	1172	OE1	GLU	157A	-28.948	66.147	-51.122	1.00	8.99	O
	ATOM	1173	OE2	GLU	157A	-27.896	65.491	-49.312	1.00	6.68	O
	ATOM	1174	C	GLU	157A	-33.773	67.161	-49.783	1.00	6.40	C
	ATOM	1175	O	GLU	157A	-34.040	68.296	-50.193	1.00	4.25	O
20	ATOM	1176	N	GLN	158A	-34.515	66.097	-50.075	1.00	5.53	N
	ATOM	1177	CA	GLN	158A	-35.717	66.226	-50.885	1.00	6.92	C
	ATOM	1178	CB	GLN	158A	-36.370	64.859	-51.093	1.00	7.82	C
	ATOM	1179	CG	GLN	158A	-37.654	64.918	-51.896	1.00	12.29	C
	ATOM	1180	CD	GLN	158A	-38.289	63.556	-52.084	1.00	14.62	C
25	ATOM	1181	OE1	GLN	158A	-38.784	62.949	-51.130	1.00	14.90	O
	ATOM	1182	NE2	GLN	158A	-38.271	63.063	-53.319	1.00	15.65	N
	ATOM	1183	C	GLN	158A	-36.681	67.140	-50.135	1.00	6.98	C
	ATOM	1184	O	GLN	158A	-37.324	68.005	-50.718	1.00	6.78	O
	ATOM	1185	N	GLU	159A	-36.771	66.932	-48.828	1.00	7.97	N
30	ATOM	1186	CA	GLU	159A	-37.649	67.733	-47.987	1.00	10.62	C
	ATOM	1187	CB	GLU	159A	-37.589	67.222	-46.550	1.00	13.43	C
	ATOM	1188	CG	GLU	159A	-38.298	68.084	-45.533	1.00	18.99	C
	ATOM	1189	CD	GLU	159A	-38.485	67.362	-44.210	1.00	22.11	C
	ATOM	1190	OE1	GLU	159A	-39.353	66.468	-44.143	1.00	23.07	O
35	ATOM	1191	OE2	GLU	159A	-37.760	67.678	-43.244	1.00	24.54	O
	ATOM	1192	C	GLU	159A	-37.274	69.210	-48.050	1.00	8.96	C
	ATOM	1193	O	GLU	159A	-38.150	70.066	-48.184	1.00	8.94	O
	ATOM	1194	N	LEU	160A	-35.980	69.507	-47.962	1.00	8.71	N
	ATOM	1195	CA	LEU	160A	-35.505	70.886	-48.021	1.00	7.21	C
40	ATOM	1196	CB	LEU	160A	-33.994	70.948	-47.764	1.00	7.92	C
	ATOM	1197	CG	LEU	160A	-33.575	70.652	-46.322	1.00	9.01	C
	ATOM	1198	CD1	LEU	160A	-32.057	70.639	-46.213	1.00	7.48	C
	ATOM	1199	CD2	LEU	160A	-34.177	71.701	-45.388	1.00	10.34	C
	ATOM	1200	C	LEU	160A	-35.835	71.532	-49.370	1.00	7.30	C
45	ATOM	1201	O	LEU	160A	-36.225	72.698	-49.419	1.00	6.22	O
	ATOM	1202	N	LEU	161A	-35.677	70.778	-50.459	1.00	6.33	N
	ATOM	1203	CA	LEU	161A	-35.990	71.304	-51.786	1.00	4.73	C
	ATOM	1204	CB	LEU	161A	-35.638	70.288	-52.882	1.00	5.96	C
	ATOM	1205	CG	LEU	161A	-36.132	70.640	-54.296	1.00	5.06	C
50	ATOM	1206	CD1	LEU	161A	-35.538	71.974	-54.753	1.00	3.20	C
	ATOM	1207	CD2	LEU	161A	-35.744	69.526	-55.259	1.00	7.06	C
	ATOM	1208	C	LEU	161A	-37.482	71.625	-51.841	1.00	3.16	C
	ATOM	1209	O	LEU	161A	-37.880	72.687	-52.317	1.00	2.71	O
	ATOM	1210	N	ASP	162A	-38.305	70.701	-51.353	1.00	2.50	N
55	ATOM	1211	CA	ASP	162A	-39.749	70.920	-51.335	1.00	5.20	C
	ATOM	1212	CB	ASP	162A	-40.471	69.799	-50.584	1.00	4.63	C
	ATOM	1213	CG	ASP	162A	-40.400	68.469	-51.293	1.00	9.21	C
	ATOM	1214	OD1	ASP	162A	-40.235	68.439	-52.532	1.00	8.31	O
	ATOM	1215	OD2	ASP	162A	-40.536	67.443	-50.597	1.00	9.33	O
60	ATOM	1216	C	ASP	162A	-40.072	72.232	-50.633	1.00	6.04	C
	ATOM	1217	O	ASP	162A	-40.942	72.996	-51.074	1.00	5.18	O

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	ATOM	1218	N	GLU	163A	-39.374	72.477	-49.528	1.00	5.27	N
	ATOM	1219	CA	GLU	163A	-39.582	73.679	-48.730	1.00	6.04	C
	ATOM	1220	CB	GLU	163A	-38.774	73.583	-47.424	1.00	9.63	C
	ATOM	1221	CG	GLU	163A	-39.178	72.401	-46.540	1.00	13.68	C
5	ATOM	1222	CD	GLU	163A	-38.327	72.256	-45.278	1.00	19.86	C
	ATOM	1223	OE1	GLU	163A	-38.519	71.266	-44.541	1.00	21.59	O
	ATOM	1224	OE2	GLU	163A	-37.471	73.125	-45.014	1.00	23.73	O
	ATOM	1225	C	GLU	163A	-39.232	74.953	-49.494	1.00	6.07	C
	ATOM	1226	O	GLU	163A	-39.989	75.925	-49.458	1.00	6.02	O
10	ATOM	1227	N	SER	164A	-38.100	74.951	-50.196	1.00	4.35	N
	ATOM	1228	CA	SER	164A	-37.700	76.124	-50.971	1.00	4.11	C
	ATOM	1229	CB	SER	164A	-36.280	75.956	-51.527	1.00	4.56	C
	ATOM	1230	OG	SER	164A	-36.241	74.976	-52.550	1.00	6.81	O
	ATOM	1231	C	SER	164A	-38.681	76.345	-52.126	1.00	4.17	C
15	ATOM	1232	O	SER	164A	-38.910	77.478	-52.552	1.00	4.06	O
	ATOM	1233	N	ILE	165A	-39.250	75.257	-52.637	1.00	4.79	N
	ATOM	1234	CA	ILE	165A	-40.219	75.348	-53.717	1.00	4.40	C
	ATOM	1235	CB	ILE	165A	-40.579	73.933	-54.259	1.00	4.84	C
	ATOM	1236	CG2	ILE	165A	-41.902	73.970	-55.026	1.00	3.37	C
20	ATOM	1237	CG1	ILE	165A	-39.436	73.434	-55.155	1.00	5.35	C
	ATOM	1238	CD1	ILE	165A	-39.633	72.044	-55.732	1.00	5.21	C
	ATOM	1239	C	ILE	165A	-41.455	76.049	-53.151	1.00	6.35	C
	ATOM	1240	O	ILE	165A	-41.991	76.979	-53.757	1.00	5.68	O
	ATOM	1241	N	ASP	166A	-41.886	75.625	-51.968	1.00	6.06	N
25	ATOM	1242	CA	ASP	166A	-43.043	76.252	-51.351	1.00	7.19	C
	ATOM	1243	CB	ASP	166A	-43.424	75.541	-50.054	1.00	10.02	C
	ATOM	1244	CG	ASP	166A	-44.725	76.061	-49.482	1.00	12.34	C
	ATOM	1245	OD1	ASP	166A	-44.708	77.091	-48.775	1.00	16.98	O
	ATOM	1246	OD2	ASP	166A	-45.770	75.452	-49.765	1.00	14.62	O
30	ATOM	1247	C	ASP	166A	-42.750	77.723	-51.080	1.00	6.93	C
	ATOM	1248	O	ASP	166A	-43.634	78.572	-51.216	1.00	6.73	O
	ATOM	1249	N	PHE	167A	-41.509	78.023	-50.697	1.00	6.69	N
	ATOM	1250	CA	PHE	167A	-41.102	79.399	-50.442	1.00	8.73	C
	ATOM	1251	CB	PHE	167A	-39.641	79.462	-49.976	1.00	11.67	C
35	ATOM	1252	CG	PHE	167A	-39.420	78.996	-48.565	1.00	15.63	C
	ATOM	1253	CD1	PHE	167A	-38.127	78.863	-48.066	1.00	17.75	C
	ATOM	1254	CD2	PHE	167A	-40.491	78.707	-47.726	1.00	18.91	C
	ATOM	1255	CE1	PHE	167A	-37.904	78.453	-46.754	1.00	19.71	C
	ATOM	1256	CE2	PHE	167A	-40.279	78.295	-46.410	1.00	20.80	C
40	ATOM	1257	CZ	PHE	167A	-38.984	78.169	-45.925	1.00	20.38	C
	ATOM	1258	C	PHE	167A	-41.245	80.191	-51.735	1.00	6.86	C
	ATOM	1259	O	PHE	167A	-41.707	81.329	-51.731	1.00	7.39	O
	ATOM	1260	N	ALA	168A	-40.824	79.584	-52.841	1.00	6.07	N
	ATOM	1261	CA	ALA	168A	-40.911	80.222	-54.153	1.00	4.85	C
45	ATOM	1262	CB	ALA	168A	-40.180	79.365	-55.202	1.00	6.74	C
	ATOM	1263	C	ALA	168A	-42.369	80.447	-54.569	1.00	3.95	C
	ATOM	1264	O	ALA	168A	-42.706	81.498	-55.115	1.00	2.50	O
	ATOM	1265	N	LEU	169A	-43.235	79.466	-54.320	1.00	2.55	N
	ATOM	1266	CA	LEU	169A	-44.640	79.620	-54.679	1.00	4.17	C
50	ATOM	1267	CB	LEU	169A	-45.428	78.330	-54.415	1.00	6.27	C
	ATOM	1268	CG	LEU	169A	-45.379	77.190	-55.446	1.00	10.90	C
	ATOM	1269	CD1	LEU	169A	-45.747	77.718	-56.828	1.00	11.75	C
	ATOM	1270	CD2	LEU	169A	-44.006	76.574	-55.477	1.00	16.93	C
	ATOM	1271	C	LEU	169A	-45.229	80.768	-53.870	1.00	2.04	C
55	ATOM	1272	O	LEU	169A	-46.162	81.436	-54.311	1.00	4.44	O
	ATOM	1273	N	GLY	170A	-44.668	81.008	-52.688	1.00	3.89	N
	ATOM	1274	CA	GLY	170A	-45.150	82.100	-51.854	1.00	3.81	C
	ATOM	1275	C	GLY	170A	-44.989	83.469	-52.502	1.00	5.02	C
	ATOM	1276	O	GLY	170A	-45.704	84.412	-52.158	1.00	6.69	O
60	ATOM	1277	N	VAL	171A	-44.043	83.602	-53.429	1.00	4.03	N
	ATOM	1278	CA	VAL	171A	-43.848	84.885	-54.093	1.00	3.30	C

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	ATOM	1279	CB	VAL	171A	-42.356	85.329	-54.083	1.00	2.32	C
	ATOM	1280	CG1	VAL	171A	-41.867	85.473	-52.654	1.00	1.00	C
	ATOM	1281	CG2	VAL	171A	-41.493	84.326	-54.845	1.00	3.30	C
5	ATOM	1282	C	VAL	171A	-44.350	84.842	-55.534	1.00	2.80	C
	ATOM	1283	O	VAL	171A	-43.896	85.604	-56.380	1.00	2.06	O
	ATOM	1284	N	LEU	172A	-45.289	83.943	-55.812	1.00	1.74	N
	ATOM	1285	CA	LEU	172A	-45.836	83.830	-57.155	1.00	2.02	C
	ATOM	1286	CB	LEU	172A	-46.876	82.707	-57.225	1.00	2.04	C
	ATOM	1287	CG	LEU	172A	-47.455	82.463	-58.618	1.00	3.25	C
10	ATOM	1288	CD1	LEU	172A	-46.328	82.116	-59.571	1.00	1.00	C
	ATOM	1289	CD2	LEU	172A	-48.478	81.331	-58.577	1.00	3.39	C
	ATOM	1290	C	LEU	172A	-46.470	85.148	-57.588	1.00	3.78	C
	ATOM	1291	O	LEU	172A	-46.305	85.579	-58.732	1.00	2.36	O
	ATOM	1292	N	PRO	173A	-47.223	85.801	-56.685	1.00	1.92	N
15	ATOM	1293	CD	PRO	173A	-47.691	85.350	-55.363	1.00	4.79	C
	ATOM	1294	CA	PRO	173A	-47.848	87.074	-57.042	1.00	4.00	C
	ATOM	1295	CB	PRO	173A	-48.461	87.532	-55.724	1.00	5.43	C
	ATOM	1296	CG	PRO	173A	-48.885	86.259	-55.108	1.00	4.83	C
	ATOM	1297	C	PRO	173A	-46.845	88.089	-57.587	1.00	3.75	C
20	ATOM	1298	O	PRO	173A	-47.121	88.790	-58.562	1.00	2.63	O
	ATOM	1299	N	GLU	174A	-45.684	88.176	-56.946	1.00	3.22	N
	ATOM	1300	CA	GLU	174A	-44.655	89.112	-57.380	1.00	4.83	C
	ATOM	1301	CB	GLU	174A	-43.550	89.236	-56.322	1.00	6.97	C
	ATOM	1302	CG	GLU	174A	-43.906	90.050	-55.076	1.00	7.95	C
25	ATOM	1303	CD	GLU	174A	-45.022	89.439	-54.255	1.00	8.81	C
	ATOM	1304	OE1	GLU	174A	-45.183	88.199	-54.283	1.00	5.68	O
	ATOM	1305	OE2	GLU	174A	-45.728	90.205	-53.563	1.00	7.94	O
	ATOM	1306	C	GLU	174A	-44.032	88.663	-58.703	1.00	4.95	C
	ATOM	1307	O	GLU	174A	-43.839	89.470	-59.609	1.00	6.20	O
30	ATOM	1308	N	MET	175A	-43.724	87.377	-58.823	1.00	5.12	N
	ATOM	1309	CA	MET	175A	-43.109	86.897	-60.058	1.00	3.89	C
	ATOM	1310	CB	MET	175A	-42.673	85.438	-59.905	1.00	2.78	C
	ATOM	1311	CG	MET	175A	-41.725	85.212	-58.747	1.00	7.50	C
	ATOM	1312	SD	MET	175A	-40.965	83.573	-58.770	1.00	7.54	S
35	ATOM	1313	CE	MET	175A	-42.374	82.552	-58.338	1.00	6.23	C
	ATOM	1314	C	MET	175A	-44.028	87.052	-61.271	1.00	2.72	C
	ATOM	1315	O	MET	175A	-43.587	87.482	-62.331	1.00	1.00	O
	ATOM	1316	N	LEU	176A	-45.303	86.706	-61.129	1.00	3.41	N
	ATOM	1317	CA	LEU	176A	-46.224	86.844	-62.251	1.00	3.01	C
40	ATOM	1318	CB	LEU	176A	-47.608	86.318	-61.871	1.00	4.67	C
	ATOM	1319	CG	LEU	176A	-47.710	84.801	-61.716	1.00	5.70	C
	ATOM	1320	CD1	LEU	176A	-49.133	84.451	-61.309	1.00	3.31	C
	ATOM	1321	CD2	LEU	176A	-47.339	84.100	-63.026	1.00	3.06	C
	ATOM	1322	C	LEU	176A	-46.332	88.298	-62.708	1.00	3.63	C
45	ATOM	1323	O	LEU	176A	-46.459	88.582	-63.901	1.00	1.32	O
	ATOM	1324	N	ALA	177A	-46.269	89.217	-61.751	1.00	3.68	N
	ATOM	1325	CA	ALA	177A	-46.361	90.639	-62.052	1.00	5.90	C
	ATOM	1326	CB	ALA	177A	-46.693	91.418	-60.781	1.00	6.48	C
	ATOM	1327	C	ALA	177A	-45.070	91.175	-62.656	1.00	5.56	C
50	ATOM	1328	O	ALA	177A	-45.073	92.215	-63.314	1.00	5.84	O
	ATOM	1329	N	GLY	178A	-43.970	90.463	-62.428	1.00	6.30	N
	ATOM	1330	CA	GLY	178A	-42.688	90.912	-62.936	1.00	5.58	C
	ATOM	1331	C	GLY	178A	-42.007	91.808	-61.918	1.00	6.25	C
	ATOM	1332	O	GLY	178A	-41.065	92.538	-62.243	1.00	6.12	O
55	ATOM	1333	N	ASP	179A	-42.485	91.751	-60.679	1.00	4.83	N
	ATOM	1334	CA	ASP	179A	-41.927	92.557	-59.594	1.00	6.18	C
	ATOM	1335	CB	ASP	179A	-43.031	92.890	-58.581	1.00	7.12	C
	ATOM	1336	CG	ASP	179A	-42.653	94.024	-57.646	1.00	8.99	C
	ATOM	1337	OD1	ASP	179A	-43.565	94.557	-56.977	1.00	8.23	O
60	ATOM	1338	OD2	ASP	179A	-41.455	94.374	-57.570	1.00	7.86	O
	ATOM	1339	C	ASP	179A	-40.791	91.780	-58.923	1.00	6.13	C

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	ATOM	1340	O	ASP	179A	-40.885	91.357	-57.771	1.00	3.72	O
	ATOM	1341	N	TRP	180A	-39.702	91.607	-59.659	1.00	7.35	N
	ATOM	1342	CA	TRP	180A	-38.554	90.863	-59.164	1.00	7.76	C
	ATOM	1343	CB	TRP	180A	-37.516	90.754	-60.278	1.00	7.58	C
5	ATOM	1344	CG	TRP	180A	-38.135	90.175	-61.506	1.00	8.22	C
	ATOM	1345	CD2	TRP	180A	-38.608	88.832	-61.671	1.00	5.10	C
	ATOM	1346	CE2	TRP	180A	-39.175	88.745	-62.957	1.00	3.86	C
	ATOM	1347	CE3	TRP	180A	-38.605	87.692	-60.851	1.00	5.18	C
	ATOM	1348	CD1	TRP	180A	-38.428	90.829	-62.666	1.00	6.12	C
10	ATOM	1349	NE1	TRP	180A	-39.053	89.977	-63.543	1.00	6.40	N
	ATOM	1350	CZ2	TRP	180A	-39.736	87.564	-63.450	1.00	3.62	C
	ATOM	1351	CZ3	TRP	180A	-39.166	86.513	-61.342	1.00	2.30	C
	ATOM	1352	CH2	TRP	180A	-39.722	86.460	-62.631	1.00	1.00	C
	ATOM	1353	C	TRP	180A	-37.944	91.451	-57.903	1.00	8.98	C
15	ATOM	1354	O	TRP	180A	-37.550	90.719	-56.996	1.00	9.71	O
	ATOM	1355	N	THR	181A	-37.875	92.773	-57.843	1.00	10.45	N
	ATOM	1356	CA	THR	181A	-37.323	93.448	-56.679	1.00	10.67	C
	ATOM	1357	CB	THR	181A	-37.522	94.959	-56.797	1.00	13.56	C
	ATOM	1358	OG1	THR	181A	-38.922	95.241	-56.895	1.00	18.11	O
20	ATOM	1359	CG2	THR	181A	-36.829	95.484	-58.041	1.00	13.17	C
	ATOM	1360	C	THR	181A	-38.017	92.953	-55.410	1.00	9.05	C
	ATOM	1361	O	THR	181A	-37.365	92.510	-54.465	1.00	7.65	O
	ATOM	1362	N	ARG	182A	-39.346	93.022	-55.404	1.00	9.67	N
	ATOM	1363	CA	ARG	182A	-40.140	92.586	-54.259	1.00	8.86	C
25	ATOM	1364	CB	ARG	182A	-41.604	92.956	-54.482	1.00	12.32	C
	ATOM	1365	CG	ARG	182A	-42.518	92.649	-53.312	1.00	16.11	C
	ATOM	1366	CD	ARG	182A	-42.265	93.565	-52.132	1.00	22.02	C
	ATOM	1367	NE	ARG	182A	-43.349	93.464	-51.159	1.00	29.18	N
	ATOM	1368	CZ	ARG	182A	-43.477	94.242	-50.090	1.00	31.14	C
30	ATOM	1369	NH1	ARG	182A	-44.502	94.071	-49.266	1.00	32.94	N
	ATOM	1370	NH2	ARG	182A	-42.583	95.192	-49.847	1.00	32.49	N
	ATOM	1371	C	ARG	182A	-40.024	91.076	-54.032	1.00	8.94	C
	ATOM	1372	O	ARG	182A	-39.887	90.614	-52.898	1.00	7.80	O
	ATOM	1373	N	ALA	183A	-40.094	90.312	-55.113	1.00	7.15	N
35	ATOM	1374	CA	ALA	183A	-39.992	88.862	-55.017	1.00	8.70	C
	ATOM	1375	CB	ALA	183A	-40.081	88.236	-56.405	1.00	9.10	C
	ATOM	1376	C	ALA	183A	-38.678	88.475	-54.350	1.00	8.80	C
	ATOM	1377	O	ALA	183A	-38.648	87.643	-53.442	1.00	7.93	O
	ATOM	1378	N	MET	184A	-37.594	89.099	-54.795	1.00	10.12	N
40	ATOM	1379	CA	MET	184A	-36.281	88.815	-54.247	1.00	10.88	C
	ATOM	1380	CB	MET	184A	-35.207	89.540	-55.060	1.00	11.54	C
	ATOM	1381	CG	MET	184A	-35.078	89.004	-56.478	1.00	14.15	C
	ATOM	1382	SD	MET	184A	-33.816	89.825	-57.464	1.00	18.18	S
	ATOM	1383	CE	MET	184A	-32.389	88.937	-56.939	1.00	17.44	C
45	ATOM	1384	C	MET	184A	-36.148	89.154	-52.765	1.00	11.97	C
	ATOM	1385	O	MET	184A	-35.515	88.416	-52.020	1.00	12.18	O
	ATOM	1386	N	GLN	185A	-36.742	90.256	-52.322	1.00	12.75	N
	ATOM	1387	CA	GLN	185A	-36.628	90.601	-50.910	1.00	15.73	C
	ATOM	1388	CB	GLN	185A	-37.011	92.065	-50.688	1.00	18.93	C
50	ATOM	1389	CG	GLN	185A	-36.159	93.022	-51.510	1.00	26.45	C
	ATOM	1390	CD	GLN	185A	-34.688	92.617	-51.538	1.00	30.92	C
	ATOM	1391	OE1	GLN	185A	-34.030	92.533	-50.498	1.00	33.71	O
	ATOM	1392	NE2	GLN	185A	-34.168	92.362	-52.737	1.00	32.42	N
	ATOM	1393	C	GLN	185A	-37.468	89.681	-50.028	1.00	14.71	C
55	ATOM	1394	O	GLN	185A	-37.107	89.398	-48.886	1.00	14.60	O
	ATOM	1395	N	LYS	186A	-38.583	89.198	-50.561	1.00	13.04	N
	ATOM	1396	CA	LYS	186A	-39.438	88.304	-49.796	1.00	13.76	C
	ATOM	1397	CB	LYS	186A	-40.857	88.289	-50.379	1.00	13.45	C
	ATOM	1398	CG	LYS	186A	-41.539	89.655	-50.367	1.00	16.00	C
60	ATOM	1399	CD	LYS	186A	-42.959	89.611	-50.908	1.00	17.17	C
	ATOM	1400	CE	LYS	186A	-43.914	88.937	-49.934	1.00	19.26	C

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	ATOM	1401	NZ	LYS	186A	-45.338	89.130	-50.333	1.00	19.39	N
	ATOM	1402	C	LYS	186A	-38.858	86.894	-49.808	1.00	14.50	C
	ATOM	1403	O	LYS	186A	-38.763	86.244	-48.767	1.00	12.91	O
	ATOM	1404	N	LEU	187A	-38.453	86.437	-50.990	1.00	14.43	N
5	ATOM	1405	CA	LEU	187A	-37.899	85.098	-51.147	1.00	16.13	C
	ATOM	1406	CB	LEU	187A	-37.796	84.747	-52.631	1.00	13.71	C
	ATOM	1407	CG	LEU	187A	-37.121	83.420	-52.985	1.00	12.09	C
	ATOM	1408	CD1	LEU	187A	-37.845	82.270	-52.305	1.00	8.66	C
	ATOM	1409	CD2	LEU	187A	-37.119	83.237	-54.496	1.00	7.97	C
	ATOM	1410	C	LEU	187A	-36.539	84.908	-50.493	1.00	18.49	C
10	ATOM	1411	O	LEU	187A	-36.348	83.988	-49.698	1.00	20.01	O
	ATOM	1412	N	HIS	188A	-35.593	85.774	-50.839	1.00	21.06	N
	ATOM	1413	CA	HIS	188A	-34.239	85.687	-50.306	1.00	23.31	C
	ATOM	1414	CB	HIS	188A	-33.366	86.777	-50.933	1.00	21.50	C
	ATOM	1415	CG	HIS	188A	-33.161	86.614	-52.409	1.00	18.52	C
	ATOM	1416	CD2	HIS	188A	-32.320	87.242	-53.265	1.00	17.87	C
15	ATOM	1417	ND1	HIS	188A	-33.894	85.730	-53.171	1.00	18.23	N
	ATOM	1418	CE1	HIS	188A	-33.514	85.821	-54.434	1.00	15.96	C
	ATOM	1419	NE2	HIS	188A	-32.562	86.732	-54.517	1.00	17.70	N
	ATOM	1420	C	HIS	188A	-34.170	85.780	-48.783	1.00	26.12	C
	ATOM	1421	O	HIS	188A	-33.149	85.443	-48.185	1.00	27.38	O
	ATOM	1422	N	SER	189A	-35.253	86.230	-48.158	1.00	29.36	N
20	ATOM	1423	CA	SER	189A	-35.286	86.359	-46.703	1.00	32.73	C
	ATOM	1424	CB	SER	189A	-36.080	87.604	-46.302	1.00	33.66	C
	ATOM	1425	OG	SER	189A	-37.427	87.506	-46.732	1.00	35.88	O
	ATOM	1426	C	SER	189A	-35.900	85.133	-46.034	1.00	33.46	C
	ATOM	1427	O	SER	189A	-36.105	85.118	-44.822	1.00	32.82	O
	ATOM	1428	N	GLN	190A	-36.189	84.107	-46.827	1.00	34.94	N
25	ATOM	1429	CA	GLN	190A	-36.782	82.877	-46.309	1.00	37.56	C
	ATOM	1430	CB	GLN	190A	-37.712	82.259	-47.354	1.00	37.62	C
	ATOM	1431	CG	GLN	190A	-38.893	83.133	-47.730	1.00	38.10	C
	ATOM	1432	CD	GLN	190A	-39.755	83.480	-46.536	1.00	38.82	C
	ATOM	1433	OE1	GLN	190A	-40.284	82.596	-45.858	1.00	37.83	O
	ATOM	1434	NE2	GLN	190A	-39.900	84.774	-46.267	1.00	38.91	N
30	ATOM	1435	C	GLN	190A	-35.712	81.862	-45.924	1.00	38.68	C
	ATOM	1436	O	GLN	190A	-34.851	81.517	-46.734	1.00	38.29	O
	ATOM	1437	N	LYS	191A	-35.775	81.384	-44.685	1.00	40.02	N
	ATOM	1438	CA	LYS	191A	-34.814	80.407	-44.184	1.00	41.24	C
	ATOM	1439	CB	LYS	191A	-34.915	79.100	-44.977	1.00	38.13	C
	ATOM	1440	CG	LYS	191A	-35.246	77.878	-44.132	1.00	33.08	C
35	ATOM	1441	CD	LYS	191A	-34.185	77.628	-43.069	1.00	29.39	C
	ATOM	1442	CE	LYS	191A	-34.557	76.454	-42.177	1.00	26.28	C
	ATOM	1443	NZ	LYS	191A	-33.506	76.170	-41.156	1.00	23.08	N
	ATOM	1444	C	LYS	191A	-33.391	80.945	-44.272	1.00	45.11	C
	ATOM	1445	O	LYS	191A	-32.432	80.244	-43.944	1.00	44.97	O
	ATOM	1446	N	ALA	192A	-33.260	82.189	-44.723	1.00	48.96	N
40	ATOM	1447	CA	ALA	192A	-31.956	82.832	-44.855	1.00	52.68	C
	ATOM	1448	CB	ALA	192A	-31.831	83.487	-46.227	1.00	52.16	C
	ATOM	1449	C	ALA	192A	-31.807	83.877	-43.755	1.00	55.30	C
	ATOM	1450	O	ALA	192A	-31.417	85.018	-44.008	1.00	55.26	O
	ATOM	1451	N	GLN	193A	-32.125	83.470	-42.530	1.00	58.43	N
	ATOM	1452	CA	GLN	193A	-32.048	84.351	-41.373	1.00	61.30	C
45	ATOM	1453	CB	GLN	193A	-33.461	84.657	-40.871	1.00	62.98	C
	ATOM	1454	CG	GLN	193A	-34.333	85.386	-41.883	1.00	65.13	C
	ATOM	1455	CD	GLN	193A	-35.797	85.391	-41.493	1.00	66.25	C
	ATOM	1456	OE1	GLN	193A	-36.158	85.809	-40.394	1.00	67.23	O
	ATOM	1457	NE2	GLN	193A	-36.651	84.923	-42.395	1.00	66.93	N
	ATOM	1458	C	GLN	193A	-31.225	83.716	-40.254	1.00	62.15	C
50	ATOM	1459	O	GLN	193A	-30.758	82.572	-40.440	1.00	62.90	O
	ATOM	1460	OXT	GLN	193A	-31.055	84.371	-39.205	1.00	63.09	O
60	TER	1461		GLN	193A						

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	ATOM	1462	CB	THR	1B	-69.713	51.046	-75.665	1.00	14.17	C
	ATOM	1463	OG1	THR	1B	-70.110	51.389	-74.332	1.00	19.10	O
	ATOM	1464	CG2	THR	1B	-70.949	50.895	-76.548	1.00	19.35	C
	ATOM	1465	C	THR	1B	-67.582	49.957	-74.944	1.00	7.70	C
5	ATOM	1466	O	THR	1B	-67.459	49.742	-73.738	1.00	4.35	O
	ATOM	1467	N	THR	1B	-69.650	48.661	-75.010	1.00	8.19	N
	ATOM	1468	CA	THR	1B	-68.912	49.735	-75.649	1.00	11.67	C
	ATOM	1469	N	ILE	2B	-66.585	50.375	-75.716	1.00	6.24	N
	ATOM	1470	CA	ILE	2B	-65.252	50.629	-75.190	1.00	7.18	C
10	ATOM	1471	CB	ILE	2B	-64.224	50.764	-76.345	1.00	9.65	C
	ATOM	1472	CG2	ILE	2B	-64.696	51.784	-77.340	1.00	10.95	C
	ATOM	1473	CG1	ILE	2B	-62.858	51.181	-75.807	1.00	11.66	C
	ATOM	1474	CD1	ILE	2B	-62.136	50.072	-75.121	1.00	15.86	C
	ATOM	1475	C	ILE	2B	-65.238	51.906	-74.359	1.00	5.63	C
15	ATOM	1476	O	ILE	2B	-65.788	52.932	-74.770	1.00	3.07	O
	ATOM	1477	N	LYS	3B	-64.612	51.830	-73.189	1.00	3.42	N
	ATOM	1478	CA	LYS	3B	-64.503	52.974	-72.293	1.00	5.41	C
	ATOM	1479	CB	LYS	3B	-65.098	52.637	-70.928	1.00	9.75	C
	ATOM	1480	CG	LYS	3B	-66.617	52.545	-70.916	1.00	15.41	C
20	ATOM	1481	CD	LYS	3B	-67.123	51.907	-69.625	1.00	20.90	C
	ATOM	1482	CE	LYS	3B	-66.687	52.692	-68.399	1.00	23.21	C
	ATOM	1483	NZ	LYS	3B	-67.036	51.980	-67.138	1.00	27.59	N
	ATOM	1484	C	LYS	3B	-63.044	53.391	-72.117	1.00	2.74	C
	ATOM	1485	O	LYS	3B	-62.756	54.472	-71.604	1.00	1.00	O
25	ATOM	1486	N	LEU	4B	-62.126	52.529	-72.540	1.00	3.03	N
	ATOM	1487	CA	LEU	4B	-60.707	52.835	-72.411	1.00	1.99	C
	ATOM	1488	CB	LEU	4B	-60.163	52.280	-71.091	1.00	1.00	C
	ATOM	1489	CG	LEU	4B	-58.657	52.462	-70.895	1.00	1.30	C
	ATOM	1490	CD1	LEU	4B	-58.348	53.950	-70.825	1.00	1.00	C
30	ATOM	1491	CD2	LEU	4B	-58.189	51.741	-69.628	1.00	1.00	C
	ATOM	1492	C	LEU	4B	-59.881	52.281	-73.565	1.00	1.97	C
	ATOM	1493	O	LEU	4B	-59.922	51.082	-73.860	1.00	1.00	O
	ATOM	1494	N	ILE	5B	-59.119	53.157	-74.208	1.00	1.00	N
	ATOM	1495	CA	ILE	5B	-58.257	52.750	-75.310	1.00	1.00	C
35	ATOM	1496	CB	ILE	5B	-58.602	53.492	-76.628	1.00	1.00	C
	ATOM	1497	CG2	ILE	5B	-57.670	53.002	-77.744	1.00	1.31	C
	ATOM	1498	CG1	ILE	5B	-60.055	53.210	-77.030	1.00	2.66	C
	ATOM	1499	CD1	ILE	5B	-60.538	54.031	-78.233	1.00	4.42	C
	ATOM	1500	C	ILE	5B	-56.826	53.067	-74.889	1.00	1.80	C
40	ATOM	1501	O	ILE	5B	-56.486	54.215	-74.611	1.00	1.00	O
	ATOM	1502	N	VAL	6B	-56.002	52.025	-74.833	1.00	1.00	N
	ATOM	1503	CA	VAL	6B	-54.613	52.140	-74.403	1.00	1.00	C
	ATOM	1504	CB	VAL	6B	-54.290	51.067	-73.329	1.00	2.83	C
	ATOM	1505	CG1	VAL	6B	-52.911	51.319	-72.753	1.00	1.00	C
45	ATOM	1506	CG2	VAL	6B	-55.340	51.069	-72.232	1.00	1.00	C
	ATOM	1507	C	VAL	6B	-53.619	51.954	-75.555	1.00	1.00	C
	ATOM	1508	O	VAL	6B	-53.769	51.041	-76.373	1.00	2.16	O
	ATOM	1509	N	GLY	7B	-52.602	52.813	-75.597	1.00	1.82	N
	ATOM	1510	CA	GLY	7B	-51.578	52.729	-76.624	1.00	1.00	C
50	ATOM	1511	C	GLY	7B	-50.235	52.481	-75.959	1.00	1.56	C
	ATOM	1512	O	GLY	7B	-49.695	53.360	-75.282	1.00	1.00	O
	ATOM	1513	N	LEU	8B	-49.698	51.282	-76.156	1.00	1.66	N
	ATOM	1514	CA	LEU	8B	-48.431	50.883	-75.558	1.00	1.00	C
	ATOM	1515	CB	LEU	8B	-48.331	49.355	-75.533	1.00	1.37	C
55	ATOM	1516	CG	LEU	8B	-49.472	48.691	-74.751	1.00	1.68	C
	ATOM	1517	CD1	LEU	8B	-49.234	47.198	-74.637	1.00	1.00	C
	ATOM	1518	CD2	LEU	8B	-49.570	49.303	-73.363	1.00	1.05	C
	ATOM	1519	C	LEU	8B	-47.193	51.469	-76.222	1.00	2.78	C
	ATOM	1520	O	LEU	8B	-47.143	51.655	-77.442	1.00	2.03	O
60	ATOM	1521	N	ALA	9B	-46.185	51.748	-75.402	1.00	2.33	N
	ATOM	1522	CA	ALA	9B	-44.937	52.316	-75.893	1.00	4.92	C

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	ATOM	1523	CB	ALA	9B	-45.224	53.591	-76.654	1.00	8.45	C
	ATOM	1524	C	ALA	9B	-44.027	52.624	-74.717	1.00	5.94	C
	ATOM	1525	O	ALA	9B	-44.456	52.564	-73.564	1.00	9.56	O
	ATOM	1526	N	ASN	10B	-42.771	52.942	-75.017	1.00	5.35	N
5	ATOM	1527	CA	ASN	10B	-41.799	53.298	-73.995	1.00	3.93	C
	ATOM	1528	CB	ASN	10B	-40.445	52.641	-74.276	1.00	2.09	C
	ATOM	1529	CG	ASN	10B	-40.378	51.204	-73.786	1.00	3.05	C
	ATOM	1530	OD1	ASN	10B	-40.486	50.940	-72.588	1.00	3.44	O
	ATOM	1531	ND2	ASN	10B	-40.182	50.269	-74.709	1.00	3.80	N
10	ATOM	1532	C	ASN	10B	-41.642	54.816	-74.011	1.00	4.25	C
	ATOM	1533	O	ASN	10B	-41.789	55.450	-75.055	1.00	3.63	O
	ATOM	1534	N	PRO	11B	-41.359	55.417	-72.846	1.00	4.95	N
	ATOM	1535	CD	PRO	11B	-41.380	54.810	-71.502	1.00	5.59	C
	ATOM	1536	CA	PRO	11B	-41.187	56.867	-72.756	1.00	6.89	C
15	ATOM	1537	CB	PRO	11B	-41.531	57.154	-71.306	1.00	6.22	C
	ATOM	1538	CG	PRO	11B	-40.959	55.965	-70.612	1.00	5.48	C
	ATOM	1539	C	PRO	11B	-39.763	57.291	-73.097	1.00	7.70	C
	ATOM	1540	O	PRO	11B	-38.855	56.462	-73.129	1.00	8.71	O
	ATOM	1541	N	GLY	12B	-39.585	58.581	-73.360	1.00	9.11	N
20	ATOM	1542	CA	GLY	12B	-38.264	59.102	-73.665	1.00	8.22	C
	ATOM	1543	C	GLY	12B	-37.940	59.282	-75.134	1.00	7.20	C
	ATOM	1544	O	GLY	12B	-38.426	58.535	-75.985	1.00	7.31	O
	ATOM	1545	N	PRO	13B	-37.093	60.270	-75.458	1.00	6.58	N
	ATOM	1546	CD	PRO	13B	-36.538	61.260	-74.514	1.00	5.90	C
25	ATOM	1547	CA	PRO	13B	-36.677	60.578	-76.825	1.00	7.02	C
	ATOM	1548	CB	PRO	13B	-35.600	61.639	-76.616	1.00	7.12	C
	ATOM	1549	CG	PRO	13B	-36.129	62.392	-75.434	1.00	4.41	C
	ATOM	1550	C	PRO	13B	-36.169	59.391	-77.640	1.00	7.04	C
	ATOM	1551	O	PRO	13B	-36.577	59.212	-78.784	1.00	7.74	O
30	ATOM	1552	N	GLU	14B	-35.299	58.580	-77.044	1.00	6.94	N
	ATOM	1553	CA	GLU	14B	-34.717	57.434	-77.746	1.00	8.06	C
	ATOM	1554	CB	GLU	14B	-33.560	56.841	-76.934	1.00	11.67	C
	ATOM	1555	CG	GLU	14B	-33.068	57.735	-75.812	1.00	18.20	C
	ATOM	1556	CD	GLU	14B	-33.949	57.647	-74.581	1.00	19.54	C
35	ATOM	1557	OE1	GLU	14B	-33.744	56.724	-73.765	1.00	22.49	O
	ATOM	1558	OE2	GLU	14B	-34.854	58.486	-74.437	1.00	18.63	O
	ATOM	1559	C	GLU	14B	-35.691	56.316	-78.113	1.00	6.25	C
	ATOM	1560	O	GLU	14B	-35.374	55.477	-78.953	1.00	6.83	O
	ATOM	1561	N	TYR	15B	-36.867	56.295	-77.497	1.00	2.91	N
40	ATOM	1562	CA	TYR	15B	-37.851	55.245	-77.785	1.00	5.46	C
	ATOM	1563	CB	TYR	15B	-38.434	54.709	-76.478	1.00	5.06	C
	ATOM	1564	CG	TYR	15B	-37.435	53.995	-75.591	1.00	5.88	C
	ATOM	1565	CD1	TYR	15B	-37.029	52.696	-75.873	1.00	6.66	C
	ATOM	1566	CE1	TYR	15B	-36.111	52.036	-75.055	1.00	7.09	C
45	ATOM	1567	CD2	TYR	15B	-36.899	54.625	-74.468	1.00	6.52	C
	ATOM	1568	CE2	TYR	15B	-35.979	53.975	-73.643	1.00	7.59	C
	ATOM	1569	CZ	TYR	15B	-35.593	52.680	-73.943	1.00	7.86	C
	ATOM	1570	OH	TYR	15B	-34.711	52.021	-73.121	1.00	9.40	O
	ATOM	1571	C	TYR	15B	-38.998	55.745	-78.653	1.00	6.23	C
50	ATOM	1572	O	TYR	15B	-39.791	54.962	-79.173	1.00	6.23	O
	ATOM	1573	N	ASP	16B	-39.074	57.060	-78.811	1.00	8.89	N
	ATOM	1574	CA	ASP	16B	-40.155	57.685	-79.557	1.00	10.79	C
	ATOM	1575	CB	ASP	16B	-39.854	59.174	-79.731	1.00	11.71	C
	ATOM	1576	CG	ASP	16B	-41.016	59.922	-80.325	1.00	9.63	C
55	ATOM	1577	OD1	ASP	16B	-42.164	59.609	-79.949	1.00	13.19	O
	ATOM	1578	OD2	ASP	16B	-40.789	60.822	-81.153	1.00	10.28	O
	ATOM	1579	C	ASP	16B	-40.630	57.097	-80.896	1.00	13.12	C
	ATOM	1580	O	ASP	16B	-41.791	56.696	-81.010	1.00	16.26	O
	ATOM	1581	N	GLN	17B	-39.766	57.038	-81.903	1.00	14.18	N
60	ATOM	1582	CA	GLN	17B	-40.185	56.518	-83.209	1.00	13.74	C
	ATOM	1583	CB	GLN	17B	-39.536	57.326	-84.338	1.00	17.09	C

	ATOM	1584	CG	GLN	17B	-39.745	58.829	-84.278	1.00	21.70	C
	ATOM	1585	CD	GLN	17B	-39.194	59.524	-85.516	1.00	25.61	C
	ATOM	1586	OE1	GLN	17B	-39.758	59.412	-86.608	1.00	27.66	O
5	ATOM	1587	NE2	GLN	17B	-38.083	60.233	-85.354	1.00	27.00	N
	ATOM	1588	C	GLN	17B	-39.871	55.041	-83.443	1.00	8.93	C
	ATOM	1589	O	GLN	17B	-39.773	54.599	-84.584	1.00	10.03	O
	ATOM	1590	N	THR	18B	-39.722	54.275	-82.373	1.00	6.09	N
	ATOM	1591	CA	THR	18B	-39.394	52.862	-82.517	1.00	3.46	C
10	ATOM	1592	CB	THR	18B	-38.623	52.364	-81.287	1.00	1.10	C
	ATOM	1593	OG1	THR	18B	-39.432	52.518	-80.117	1.00	1.00	O
	ATOM	1594	CG2	THR	18B	-37.330	53.169	-81.123	1.00	2.22	C
	ATOM	1595	C	THR	18B	-40.628	51.997	-82.763	1.00	1.90	C
	ATOM	1596	O	THR	18B	-41.752	52.388	-82.461	1.00	1.00	O
15	ATOM	1597	N	ARG	19B	-40.416	50.817	-83.329	1.00	1.60	N
	ATOM	1598	CA	ARG	19B	-41.532	49.931	-83.640	1.00	1.45	C
	ATOM	1599	CB	ARG	19B	-41.022	48.628	-84.281	1.00	1.38	C
	ATOM	1600	CG	ARG	19B	-40.241	48.795	-85.593	1.00	1.00	C
	ATOM	1601	CD	ARG	19B	-39.635	47.455	-86.019	1.00	2.24	C
20	ATOM	1602	NE	ARG	19B	-39.111	47.460	-87.381	1.00	3.25	N
	ATOM	1603	CZ	ARG	19B	-37.820	47.466	-87.703	1.00	3.40	C
	ATOM	1604	NH1	ARG	19B	-36.882	47.471	-86.764	1.00	5.26	N
	ATOM	1605	NH2	ARG	19B	-37.466	47.454	-88.979	1.00	3.20	N
	ATOM	1606	C	ARG	19B	-42.393	49.581	-82.427	1.00	1.00	C
25	ATOM	1607	O	ARG	19B	-43.616	49.498	-82.536	1.00	1.56	O
	ATOM	1608	N	HIS	20B	-41.751	49.381	-81.280	1.00	1.00	N
	ATOM	1609	CA	HIS	20B	-42.443	48.995	-80.049	1.00	1.00	C
	ATOM	1610	CB	HIS	20B	-41.398	48.715	-78.961	1.00	3.27	C
	ATOM	1611	CG	HIS	20B	-41.756	47.591	-78.036	1.00	2.12	C
30	ATOM	1612	CD2	HIS	20B	-41.487	47.400	-76.722	1.00	4.27	C
	ATOM	1613	ND1	HIS	20B	-42.428	46.463	-78.453	1.00	1.93	N
	ATOM	1614	CE1	HIS	20B	-42.559	45.628	-77.438	1.00	2.29	C
	ATOM	1615	NE2	HIS	20B	-41.996	46.172	-76.376	1.00	1.00	N
	ATOM	1616	C	HIS	20B	-43.468	50.036	-79.573	1.00	1.00	C
35	ATOM	1617	O	HIS	20B	-44.311	49.736	-78.735	1.00	1.00	O
	ATOM	1618	N	ASN	21B	-43.394	51.253	-80.112	1.00	1.26	N
	ATOM	1619	CA	ASN	21B	-44.325	52.322	-79.741	1.00	1.00	C
	ATOM	1620	CB	ASN	21B	-43.593	53.666	-79.686	1.00	3.79	C
	ATOM	1621	CG	ASN	21B	-42.853	53.867	-78.383	1.00	1.84	C
40	ATOM	1622	OD1	ASN	21B	-42.378	52.909	-77.774	1.00	1.00	O
	ATOM	1623	ND2	ASN	21B	-42.757	55.113	-77.944	1.00	5.23	N
	ATOM	1624	C	ASN	21B	-45.493	52.412	-80.718	1.00	1.76	C
	ATOM	1625	O	ASN	21B	-46.187	53.423	-80.780	1.00	2.54	O
	ATOM	1626	N	ALA	22B	-45.704	51.341	-81.477	1.00	3.67	N
	ATOM	1627	CA	ALA	22B	-46.778	51.290	-82.461	1.00	1.00	C
45	ATOM	1628	CB	ALA	22B	-46.857	49.889	-83.064	1.00	1.00	C
	ATOM	1629	C	ALA	22B	-48.134	51.682	-81.874	1.00	1.00	C
	ATOM	1630	O	ALA	22B	-48.857	52.504	-82.450	1.00	4.52	O
	ATOM	1631	N	GLY	23B	-48.462	51.093	-80.728	1.00	1.52	N
	ATOM	1632	CA	GLY	23B	-49.732	51.352	-80.070	1.00	1.96	C
50	ATOM	1633	C	GLY	23B	-49.922	52.789	-79.632	1.00	2.74	C
	ATOM	1634	O	GLY	23B	-50.999	53.361	-79.811	1.00	2.44	O
	ATOM	1635	N	ALA	24B	-48.877	53.371	-79.049	1.00	1.00	N
	ATOM	1636	CA	ALA	24B	-48.934	54.752	-78.592	1.00	1.25	C
	ATOM	1637	CB	ALA	24B	-47.639	55.117	-77.881	1.00	1.00	C
55	ATOM	1638	C	ALA	24B	-49.162	55.677	-79.781	1.00	1.62	C
	ATOM	1639	O	ALA	24B	-49.857	56.693	-79.668	1.00	1.62	O
	ATOM	1640	N	LEU	25B	-48.574	55.328	-80.922	1.00	2.04	N
	ATOM	1641	CA	LEU	25B	-48.737	56.134	-82.131	1.00	3.30	C
	ATOM	1642	CB	LEU	25B	-47.872	55.575	-83.272	1.00	3.24	C
60	ATOM	1643	CG	LEU	25B	-47.965	56.306	-84.619	1.00	3.57	C
	ATOM	1644	CD1	LEU	25B	-47.715	57.795	-84.442	1.00	4.11	C

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	ATOM	1645	CD2	LEU	25B	-46.946	55.713	-85.582	1.00	3.61	C
	ATOM	1646	C	LEU	25B	-50.206	56.114	-82.532	1.00	3.74	C
	ATOM	1647	O	LEU	25B	-50.771	57.135	-82.932	1.00	2.20	O
	ATOM	1648	N	PHE	26B	-50.823	54.943	-82.419	1.00	3.68	N
5	ATOM	1649	CA	PHE	26B	-52.230	54.802	-82.752	1.00	3.09	C
	ATOM	1650	CB	PHE	26B	-52.714	53.369	-82.506	1.00	2.74	C
	ATOM	1651	CG	PHE	26B	-54.204	53.214	-82.637	1.00	2.57	C
	ATOM	1652	CD1	PHE	26B	-54.818	53.339	-83.878	1.00	2.58	C
	ATOM	1653	CD2	PHE	26B	-54.999	53.003	-81.516	1.00	3.15	C
	ATOM	1654	CE1	PHE	26B	-56.206	53.262	-84.004	1.00	1.71	C
10	ATOM	1655	CE2	PHE	26B	-56.392	52.925	-81.631	1.00	4.52	C
	ATOM	1656	CZ	PHE	26B	-56.992	53.055	-82.874	1.00	1.00	C
	ATOM	1657	C	PHE	26B	-53.074	55.749	-81.902	1.00	2.36	C
	ATOM	1658	O	PHE	26B	-53.903	56.487	-82.428	1.00	1.89	O
	ATOM	1659	N	VAL	27B	-52.869	55.712	-80.587	1.00	1.00	N
	ATOM	1660	CA	VAL	27B	-53.630	56.565	-79.679	1.00	1.00	C
15	ATOM	1661	CB	VAL	27B	-53.346	56.212	-78.211	1.00	1.00	C
	ATOM	1662	CG1	VAL	27B	-54.162	57.122	-77.280	1.00	1.00	C
	ATOM	1663	CG2	VAL	27B	-53.704	54.756	-77.964	1.00	1.00	C
	ATOM	1664	C	VAL	27B	-53.327	58.037	-79.913	1.00	1.00	C
	ATOM	1665	O	VAL	27B	-54.202	58.887	-79.756	1.00	1.00	O
	ATOM	1666	N	GLU	28B	-52.092	58.342	-80.299	1.00	1.51	N
20	ATOM	1667	CA	GLU	28B	-51.732	59.725	-80.571	1.00	1.56	C
	ATOM	1668	CB	GLU	28B	-50.220	59.851	-80.812	1.00	1.61	C
	ATOM	1669	CG	GLU	28B	-49.777	61.235	-81.254	1.00	5.63	C
	ATOM	1670	CD	GLU	28B	-48.362	61.567	-80.797	1.00	4.97	C
	ATOM	1671	OE1	GLU	28B	-47.465	60.715	-80.950	1.00	5.58	O
	ATOM	1672	OE2	GLU	28B	-48.149	62.683	-80.292	1.00	12.07	O
25	ATOM	1673	C	GLU	28B	-52.508	60.231	-81.783	1.00	3.14	C
	ATOM	1674	O	GLU	28B	-52.994	61.364	-81.785	1.00	4.58	O
	ATOM	1675	N	ARG	29B	-52.631	59.388	-82.805	1.00	1.00	N
	ATOM	1676	CA	ARG	29B	-53.356	59.752	-84.021	1.00	2.54	C
	ATOM	1677	CB	ARG	29B	-53.181	58.683	-85.099	1.00	4.85	C
	ATOM	1678	CG	ARG	29B	-51.798	58.592	-85.708	1.00	7.81	C
30	ATOM	1679	CD	ARG	29B	-51.457	59.822	-86.526	1.00	10.14	C
	ATOM	1680	NE	ARG	29B	-50.211	59.632	-87.261	1.00	9.57	N
	ATOM	1681	CZ	ARG	29B	-49.675	60.524	-88.086	1.00	9.57	C
	ATOM	1682	NH1	ARG	29B	-50.269	61.692	-88.292	1.00	10.92	N
	ATOM	1683	NH2	ARG	29B	-48.549	60.235	-88.723	1.00	11.35	N
	ATOM	1684	C	ARG	29B	-54.841	59.888	-83.730	1.00	1.00	C
35	ATOM	1685	O	ARG	29B	-55.494	60.842	-84.165	1.00	1.83	O
	ATOM	1686	N	LEU	30B	-55.367	58.908	-83.005	1.00	1.51	N
	ATOM	1687	CA	LEU	30B	-56.776	58.880	-82.636	1.00	2.09	C
	ATOM	1688	CB	LEU	30B	-57.050	57.655	-81.765	1.00	2.62	C
	ATOM	1689	CG	LEU	30B	-58.469	57.088	-81.674	1.00	1.09	C
	ATOM	1690	CD1	LEU	30B	-58.575	56.297	-80.380	1.00	6.99	C
40	ATOM	1691	CD2	LEU	30B	-59.528	58.175	-81.710	1.00	4.33	C
	ATOM	1692	C	LEU	30B	-57.125	60.142	-81.852	1.00	3.33	C
	ATOM	1693	O	LEU	30B	-58.121	60.820	-82.138	1.00	1.00	O
	ATOM	1694	N	ALA	31B	-56.289	60.458	-80.867	1.00	2.74	N
	ATOM	1695	CA	ALA	31B	-56.518	61.625	-80.028	1.00	2.12	C
	ATOM	1696	CB	ALA	31B	-55.438	61.723	-78.964	1.00	4.15	C
45	ATOM	1697	C	ALA	31B	-56.562	62.902	-80.857	1.00	2.84	C
	ATOM	1698	O	ALA	31B	-57.458	63.721	-80.692	1.00	1.00	O
	ATOM	1699	N	HIS	32B	-55.604	63.072	-81.761	1.00	2.81	N
	ATOM	1700	CA	HIS	32B	-55.599	64.275	-82.578	1.00	3.23	C
	ATOM	1701	CB	HIS	32B	-54.322	64.361	-83.420	1.00	4.61	C
	ATOM	1702	CG	HIS	32B	-54.148	65.686	-84.091	1.00	7.18	C
50	ATOM	1703	CD2	HIS	32B	-53.778	66.889	-83.595	1.00	9.15	C
	ATOM	1704	ND1	HIS	32B	-54.455	65.897	-85.417	1.00	8.21	N
55	ATOM	1705	CE1	HIS	32B	-54.284	67.173	-85.709	1.00	9.96	C

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	ATOM	1706	NE2	HIS	32B	-53.874	67.798	-84.620	1.00	10.09	N
	ATOM	1707	C	HIS	32B	-56.832	64.323	-83.483	1.00	3.91	C
	ATOM	1708	O	HIS	32B	-57.463	65.370	-83.630	1.00	4.32	O
	ATOM	1709	N	ALA	33B	-57.180	63.183	-84.068	1.00	5.05	N
5	ATOM	1710	CA	ALA	33B	-58.333	63.108	-84.950	1.00	4.34	C
	ATOM	1711	CB	ALA	33B	-58.388	61.738	-85.607	1.00	2.99	C
	ATOM	1712	C	ALA	33B	-59.648	63.404	-84.224	1.00	6.03	C
	ATOM	1713	O	ALA	33B	-60.606	63.879	-84.839	1.00	4.20	O
	ATOM	1714	N	GLN	34B	-59.696	63.149	-82.918	1.00	5.65	N
	ATOM	1715	CA	GLN	34B	-60.918	63.393	-82.153	1.00	7.73	C
10	ATOM	1716	CB	GLN	34B	-61.200	62.225	-81.201	1.00	6.98	C
	ATOM	1717	CG	GLN	34B	-61.532	60.906	-81.884	1.00	9.48	C
	ATOM	1718	CD	GLN	34B	-62.715	61.013	-82.833	1.00	11.18	C
	ATOM	1719	OE1	GLN	34B	-63.739	61.610	-82.504	1.00	13.98	O
15	ATOM	1720	NE2	GLN	34B	-62.576	60.433	-84.016	1.00	13.79	N
	ATOM	1721	C	GLN	34B	-60.888	64.697	-81.353	1.00	6.05	C
	ATOM	1722	O	GLN	34B	-61.829	64.989	-80.608	1.00	4.70	O
	ATOM	1723	N	GLY	35B	-59.810	65.461	-81.507	1.00	4.94	N
	ATOM	1724	CA	GLY	35B	-59.669	66.727	-80.802	1.00	6.33	C
20	ATOM	1725	C	GLY	35B	-59.446	66.575	-79.306	1.00	7.40	C
	ATOM	1726	O	GLY	35B	-59.812	67.455	-78.523	1.00	6.28	O
	ATOM	1727	N	VAL	36B	-58.823	65.470	-78.907	1.00	4.97	N
	ATOM	1728	CA	VAL	36B	-58.568	65.198	-77.497	1.00	4.13	C
25	ATOM	1729	CB	VAL	36B	-58.809	63.703	-77.174	1.00	3.38	C
	ATOM	1730	CG1	VAL	36B	-58.464	63.414	-75.717	1.00	2.16	C
	ATOM	1731	CG2	VAL	36B	-60.251	63.335	-77.477	1.00	3.47	C
	ATOM	1732	C	VAL	36B	-57.156	65.543	-77.029	1.00	4.02	C
	ATOM	1733	O	VAL	36B	-56.166	65.129	-77.639	1.00	4.44	O
30	ATOM	1734	N	SER	37B	-57.073	66.288	-75.933	1.00	4.82	N
	ATOM	1735	CA	SER	37B	-55.785	66.655	-75.354	1.00	6.56	C
	ATOM	1736	CB	SER	37B	-55.896	67.968	-74.568	1.00	8.88	C
	ATOM	1737	OG	SER	37B	-56.163	69.069	-75.410	1.00	10.31	O
	ATOM	1738	C	SER	37B	-55.347	65.554	-74.390	1.00	5.80	C
35	ATOM	1739	O	SER	37B	-56.124	65.132	-73.539	1.00	6.42	O
	ATOM	1740	N	LEU	38B	-54.104	65.099	-74.509	1.00	5.09	N
	ATOM	1741	CA	LEU	38B	-53.594	64.070	-73.608	1.00	5.24	C
	ATOM	1742	CB	LEU	38B	-52.635	63.126	-74.346	1.00	7.30	C
	ATOM	1743	CG	LEU	38B	-53.228	62.242	-75.447	1.00	8.28	C
40	ATOM	1744	CD1	LEU	38B	-54.372	61.410	-74.871	1.00	7.41	C
	ATOM	1745	CD2	LEU	38B	-53.722	63.109	-76.595	1.00	12.19	C
	ATOM	1746	C	LEU	38B	-52.863	64.757	-72.452	1.00	4.62	C
	ATOM	1747	O	LEU	38B	-51.795	65.339	-72.640	1.00	5.24	O
	ATOM	1748	N	VAL	39B	-53.444	64.688	-71.260	1.00	4.32	N
45	ATOM	1749	CA	VAL	39B	-52.850	65.319	-70.085	1.00	4.16	C
	ATOM	1750	CB	VAL	39B	-53.947	65.818	-69.105	1.00	3.31	C
	ATOM	1751	CG1	VAL	39B	-54.900	64.693	-68.767	1.00	8.08	C
	ATOM	1752	CG2	VAL	39B	-53.316	66.343	-67.827	1.00	1.03	C
	ATOM	1753	C	VAL	39B	-51.913	64.389	-69.323	1.00	2.61	C
50	ATOM	1754	O	VAL	39B	-52.225	63.213	-69.102	1.00	2.82	O
	ATOM	1755	N	ALA	40B	-50.749	64.913	-68.952	1.00	2.98	N
	ATOM	1756	CA	ALA	40B	-49.798	64.139	-68.172	1.00	1.00	C
	ATOM	1757	CB	ALA	40B	-48.428	64.836	-68.142	1.00	1.00	C
	ATOM	1758	C	ALA	40B	-50.403	64.095	-66.774	1.00	3.09	C
55	ATOM	1759	O	ALA	40B	-50.524	65.128	-66.109	1.00	4.80	O
	ATOM	1760	N	ASP	41B	-50.812	62.908	-66.343	1.00	2.70	N
	ATOM	1761	CA	ASP	41B	-51.398	62.746	-65.024	1.00	2.73	C
	ATOM	1762	CB	ASP	41B	-52.880	62.367	-65.128	1.00	2.19	C
	ATOM	1763	CG	ASP	41B	-53.615	62.547	-63.812	1.00	3.33	C
60	ATOM	1764	OD1	ASP	41B	-52.961	62.442	-62.758	1.00	4.07	O
	ATOM	1765	OD2	ASP	41B	-54.841	62.785	-63.826	1.00	2.44	O
	ATOM	1766	C	ASP	41B	-50.639	61.653	-64.281	1.00	2.67	C

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	ATOM	1767	O	ASP	41B	-50.751	60.470	-64.597	1.00	1.11	O
	ATOM	1768	N	ARG	42B	-49.861	62.054	-63.288	1.00	5.24	N
	ATOM	1769	CA	ARG	42B	-49.071	61.098	-62.528	1.00	5.50	C
	ATOM	1770	CB	ARG	42B	-48.143	61.845	-61.580	1.00	5.82	C
5	ATOM	1771	CG	ARG	42B	-47.478	63.021	-62.262	1.00	11.39	C
	ATOM	1772	CD	ARG	42B	-46.223	63.437	-61.546	1.00	14.28	C
	ATOM	1773	NE	ARG	42B	-45.410	62.285	-61.213	1.00	14.25	N
	ATOM	1774	CZ	ARG	42B	-44.098	62.323	-61.038	1.00	14.47	C
	ATOM	1775	NH1	ARG	42B	-43.440	63.468	-61.167	1.00	11.91	N
10	ATOM	1776	NH2	ARG	42B	-43.449	61.211	-60.740	1.00	10.85	N
	ATOM	1777	C	ARG	42B	-49.925	60.102	-61.758	1.00	3.65	C
	ATOM	1778	O	ARG	42B	-49.463	59.007	-61.429	1.00	3.77	O
	ATOM	1779	N	LYS	43B	-51.170	60.472	-61.474	1.00	3.86	N
	ATOM	1780	CA	LYS	43B	-52.057	59.577	-60.748	1.00	4.12	C
15	ATOM	1781	CB	LYS	43B	-53.373	60.276	-60.407	1.00	4.21	C
	ATOM	1782	CG	LYS	43B	-53.214	61.463	-59.473	1.00	5.72	C
	ATOM	1783	CD	LYS	43B	-54.556	61.890	-58.908	1.00	10.20	C
	ATOM	1784	CE	LYS	43B	-55.466	62.420	-59.992	1.00	10.59	C
	ATOM	1785	NZ	LYS	43B	-54.933	63.701	-60.527	1.00	16.01	N
20	ATOM	1786	C	LYS	43B	-52.338	58.324	-61.564	1.00	3.86	C
	ATOM	1787	O	LYS	43B	-52.743	57.300	-61.017	1.00	4.67	O
	ATOM	1788	N	TYR	44B	-52.112	58.400	-62.873	1.00	4.13	N
	ATOM	1789	CA	TYR	44B	-52.361	57.251	-63.744	1.00	3.46	C
	ATOM	1790	CB	TYR	44B	-53.511	57.569	-64.694	1.00	2.46	C
25	ATOM	1791	CG	TYR	44B	-54.730	58.049	-63.946	1.00	4.57	C
	ATOM	1792	CD1	TYR	44B	-55.114	59.384	-63.991	1.00	6.35	C
	ATOM	1793	CE1	TYR	44B	-56.202	59.848	-63.253	1.00	7.49	C
	ATOM	1794	CD2	TYR	44B	-55.466	57.174	-63.145	1.00	5.10	C
	ATOM	1795	CE2	TYR	44B	-56.554	57.623	-62.404	1.00	5.75	C
30	ATOM	1796	CZ	TYR	44B	-56.915	58.962	-62.464	1.00	9.05	C
	ATOM	1797	OH	TYR	44B	-57.997	59.408	-61.743	1.00	9.39	O
	ATOM	1798	C	TYR	44B	-51.128	56.822	-64.525	1.00	1.98	C
	ATOM	1799	O	TYR	44B	-51.222	56.107	-65.523	1.00	1.00	O
	ATOM	1800	N	PHE	45B	-49.969	57.276	-64.060	1.00	2.20	N
35	ATOM	1801	CA	PHE	45B	-48.697	56.911	-64.674	1.00	1.00	C
	ATOM	1802	CB	PHE	45B	-48.322	55.486	-64.240	1.00	2.60	C
	ATOM	1803	CG	PHE	45B	-48.355	55.283	-62.749	1.00	3.03	C
	ATOM	1804	CD1	PHE	45B	-47.352	55.808	-61.937	1.00	5.79	C
	ATOM	1805	CD2	PHE	45B	-49.415	54.617	-62.149	1.00	4.76	C
40	ATOM	1806	CE1	PHE	45B	-47.412	55.676	-60.550	1.00	6.82	C
	ATOM	1807	CE2	PHE	45B	-49.483	54.480	-60.760	1.00	6.40	C
	ATOM	1808	CZ	PHE	45B	-48.481	55.012	-59.961	1.00	5.39	C
	ATOM	1809	C	PHE	45B	-48.742	57.003	-66.197	1.00	2.63	C
	ATOM	1810	O	PHE	45B	-48.285	56.097	-66.898	1.00	2.11	O
45	ATOM	1811	N	GLY	46B	-49.290	58.099	-66.712	1.00	1.00	N
	ATOM	1812	CA	GLY	46B	-49.343	58.245	-68.152	1.00	3.06	C
	ATOM	1813	C	GLY	46B	-50.063	59.480	-68.642	1.00	2.11	C
	ATOM	1814	O	GLY	46B	-50.411	60.360	-67.859	1.00	1.94	O
	ATOM	1815	N	LEU	47B	-50.273	59.528	-69.953	1.00	2.30	N
50	ATOM	1816	CA	LEU	47B	-50.962	60.625	-70.615	1.00	1.51	C
	ATOM	1817	CB	LEU	47B	-50.349	60.862	-71.987	1.00	1.00	C
	ATOM	1818	CG	LEU	47B	-48.825	61.008	-72.018	1.00	2.31	C
	ATOM	1819	CD1	LEU	47B	-48.375	61.271	-73.452	1.00	2.89	C
	ATOM	1820	CD2	LEU	47B	-48.387	62.134	-71.100	1.00	1.00	C
55	ATOM	1821	C	LEU	47B	-52.406	60.184	-70.779	1.00	1.30	C
	ATOM	1822	O	LEU	47B	-52.664	59.146	-71.377	1.00	2.38	O
	ATOM	1823	N	VAL	48B	-53.345	60.970	-70.263	1.00	2.03	N
	ATOM	1824	CA	VAL	48B	-54.750	60.593	-70.342	1.00	2.91	C
	ATOM	1825	CB	VAL	48B	-55.308	60.272	-68.937	1.00	5.58	C
60	ATOM	1826	CG1	VAL	48B	-54.454	59.193	-68.272	1.00	7.03	C
	ATOM	1827	CG2	VAL	48B	-55.342	61.525	-68.085	1.00	10.71	C

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	ATOM	1828	C	VAL	48B	-55.649	61.632	-70.994	1.00	2.78
	ATOM	1829	O	VAL	48B	-55.549	62.821	-70.714	1.00	1.09
	ATOM	1830	N	GLY	49B	-56.528	61.160	-71.870	1.00	3.32
	ATOM	1831	CA	GLY	49B	-57.453	62.041	-72.556	1.00	4.17
5	ATOM	1832	C	GLY	49B	-58.874	61.525	-72.427	1.00	4.82
	ATOM	1833	O	GLY	49B	-59.097	60.373	-72.064	1.00	1.00
	ATOM	1834	N	LYS	50B	-59.849	62.363	-72.734	1.00	5.07
	ATOM	1835	CA	LYS	50B	-61.230	61.922	-72.614	1.00	7.20
	ATOM	1836	CB	LYS	50B	-61.723	62.184	-71.185	1.00	10.55
10	ATOM	1837	CG	LYS	50B	-63.106	61.644	-70.876	1.00	16.38
	ATOM	1838	CD	LYS	50B	-63.554	62.041	-69.474	1.00	20.00
	ATOM	1839	CE	LYS	50B	-63.640	63.555	-69.323	1.00	23.07
	ATOM	1840	NZ	LYS	50B	-64.114	63.960	-67.969	1.00	25.94
	ATOM	1841	C	LYS	50B	-62.134	62.631	-73.605	1.00	5.78
15	ATOM	1842	O	LYS	50B	-61.916	63.797	-73.919	1.00	3.76
	ATOM	1843	N	PHE	51B	-63.127	61.906	-74.114	1.00	6.08
	ATOM	1844	CA	PHE	51B	-64.109	62.470	-75.030	1.00	7.04
	ATOM	1845	CB	PHE	51B	-63.656	62.401	-76.501	1.00	8.44
	ATOM	1846	CG	PHE	51B	-63.477	61.012	-77.042	1.00	9.37
20	ATOM	1847	CD1	PHE	51B	-62.389	60.234	-76.664	1.00	11.86
	ATOM	1848	CD2	PHE	51B	-64.375	60.500	-77.981	1.00	11.25
	ATOM	1849	CE1	PHE	51B	-62.190	58.965	-77.213	1.00	10.65
	ATOM	1850	CE2	PHE	51B	-64.188	59.233	-78.537	1.00	11.95
	ATOM	1851	CZ	PHE	51B	-63.090	58.463	-78.153	1.00	12.36
25	ATOM	1852	C	PHE	51B	-65.419	61.727	-74.832	1.00	8.35
	ATOM	1853	O	PHE	51B	-65.464	60.707	-74.143	1.00	5.80
	ATOM	1854	N	SER	52B	-66.488	62.244	-75.420	1.00	7.02
	ATOM	1855	CA	SER	52B	-67.782	61.611	-75.264	1.00	8.60
	ATOM	1856	CB	SER	52B	-68.837	62.655	-74.892	1.00	11.89
30	ATOM	1857	OG	SER	52B	-68.501	63.313	-73.686	1.00	16.40
	ATOM	1858	C	SER	52B	-68.233	60.889	-76.512	1.00	7.29
	ATOM	1859	O	SER	52B	-67.967	61.329	-77.624	1.00	7.38
	ATOM	1860	N	HIS	53B	-68.916	59.767	-76.318	1.00	6.91
	ATOM	1861	CA	HIS	53B	-69.451	59.007	-77.427	1.00	4.00
35	ATOM	1862	CB	HIS	53B	-68.468	57.969	-77.939	1.00	6.50
	ATOM	1863	CG	HIS	53B	-68.874	57.385	-79.253	1.00	4.95
	ATOM	1864	CD2	HIS	53B	-68.974	57.950	-80.480	1.00	2.78
	ATOM	1865	ND1	HIS	53B	-69.283	56.078	-79.398	1.00	4.84
	ATOM	1866	CE1	HIS	53B	-69.615	55.862	-80.658	1.00	4.28
40	ATOM	1867	NE2	HIS	53B	-69.437	56.981	-81.335	1.00	5.11
	ATOM	1868	C	HIS	53B	-70.720	58.307	-76.989	1.00	5.23
	ATOM	1869	O	HIS	53B	-70.710	57.521	-76.044	1.00	2.21
	ATOM	1870	N	GLN	54B	-71.809	58.596	-77.691	1.00	5.12
	ATOM	1871	CA	GLN	54B	-73.106	58.013	-77.363	1.00	5.26
45	ATOM	1872	CB	GLN	54B	-73.153	56.531	-77.770	1.00	4.22
	ATOM	1873	CG	GLN	54B	-73.133	56.350	-79.285	1.00	4.12
	ATOM	1874	CD	GLN	54B	-73.215	54.902	-79.741	1.00	5.05
	ATOM	1875	OE1	GLN	54B	-73.112	54.620	-80.933	1.00	6.32
	ATOM	1876	NE2	GLN	54B	-73.409	53.982	-78.801	1.00	4.68
50	ATOM	1877	C	GLN	54B	-73.412	58.184	-75.877	1.00	5.51
	ATOM	1878	O	GLN	54B	-73.874	57.256	-75.203	1.00	8.09
	ATOM	1879	N	GLY	55B	-73.144	59.390	-75.383	1.00	6.38
	ATOM	1880	CA	GLY	55B	-73.416	59.731	-73.998	1.00	9.07
	ATOM	1881	C	GLY	55B	-72.532	59.131	-72.925	1.00	8.91
55	ATOM	1882	O	GLY	55B	-72.811	59.308	-71.741	1.00	8.26
	ATOM	1883	N	ALA	56B	-71.479	58.421	-73.320	1.00	10.25
	ATOM	1884	CA	ALA	56B	-70.574	57.811	-72.352	1.00	9.81
	ATOM	1885	CB	ALA	56B	-70.558	56.298	-72.525	1.00	9.66
	ATOM	1886	C	ALA	56B	-69.163	58.373	-72.509	1.00	9.36
60	ATOM	1887	O	ALA	56B	-68.784	58.834	-73.586	1.00	9.03
	ATOM	1888	N	ASP	57B	-68.397	58.344	-71.423	1.00	10.54

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	ATOM	1889	CA	ASP	57B	-67.023	58.838	-71.424	1.00	8.91	C
	ATOM	1890	CB	ASP	57B	-66.601	59.241	-70.007	1.00	11.75	C
	ATOM	1891	CG	ASP	57B	-67.172	60.583	-69.576	1.00	14.68	C
	ATOM	1892	OD1	ASP	57B	-67.158	60.863	-68.361	1.00	16.93	O
5	ATOM	1893	OD2	ASP	57B	-67.612	61.364	-70.443	1.00	17.83	O
	ATOM	1894	C	ASP	57B	-66.063	57.766	-71.929	1.00	8.22	C
	ATOM	1895	O	ASP	57B	-66.042	56.656	-71.399	1.00	8.19	O
	ATOM	1896	N	VAL	58B	-65.278	58.100	-72.952	1.00	5.00	N
	ATOM	1897	CA	VAL	58B	-64.291	57.175	-73.504	1.00	4.89	C
10	ATOM	1898	CB	VAL	58B	-64.487	56.974	-75.029	1.00	2.61	C
	ATOM	1899	CG1	VAL	58B	-63.493	55.945	-75.558	1.00	2.22	C
	ATOM	1900	CG2	VAL	58B	-65.912	56.526	-75.312	1.00	1.51	C
	ATOM	1901	C	VAL	58B	-62.906	57.775	-73.249	1.00	4.92	C
	ATOM	1902	O	VAL	58B	-62.611	58.872	-73.711	1.00	5.02	O
15	ATOM	1903	N	ARG	59B	-62.059	57.058	-72.517	1.00	3.99	N
	ATOM	1904	CA	ARG	59B	-60.727	57.570	-72.207	1.00	4.16	C
	ATOM	1905	CB	ARG	59B	-60.440	57.395	-70.708	1.00	3.88	C
	ATOM	1906	CG	ARG	59B	-61.364	58.252	-69.840	1.00	5.88	C
	ATOM	1907	CD	ARG	59B	-61.009	58.248	-68.356	1.00	5.75	C
20	ATOM	1908	NE	ARG	59B	-61.755	59.286	-67.636	1.00	9.77	N
	ATOM	1909	CZ	ARG	59B	-63.070	59.262	-67.426	1.00	11.45	C
	ATOM	1910	NH1	ARG	59B	-63.663	60.254	-66.768	1.00	10.65	N
	ATOM	1911	NH2	ARG	59B	-63.798	58.241	-67.864	1.00	9.53	N
	ATOM	1912	C	ARG	59B	-59.593	56.974	-73.033	1.00	2.95	C
25	ATOM	1913	O	ARG	59B	-59.634	55.805	-73.434	1.00	1.00	O
	ATOM	1914	N	LEU	60B	-58.597	57.816	-73.297	1.00	2.31	N
	ATOM	1915	CA	LEU	60B	-57.418	57.441	-74.062	1.00	1.50	C
	ATOM	1916	CB	LEU	60B	-57.202	58.431	-75.209	1.00	1.00	C
	ATOM	1917	CG	LEU	60B	-58.380	58.575	-76.179	1.00	2.84	C
30	ATOM	1918	CD1	LEU	60B	-58.042	59.561	-77.278	1.00	4.61	C
	ATOM	1919	CD2	LEU	60B	-58.704	57.207	-76.769	1.00	1.72	C
	ATOM	1920	C	LEU	60B	-56.220	57.454	-73.116	1.00	2.09	C
	ATOM	1921	O	LEU	60B	-56.018	58.407	-72.360	1.00	1.82	O
	ATOM	1922	N	LEU	61B	-55.421	56.397	-73.160	1.00	1.00	N
35	ATOM	1923	CA	LEU	61B	-54.276	56.301	-72.271	1.00	1.00	C
	ATOM	1924	CB	LEU	61B	-54.595	55.336	-71.121	1.00	2.58	C
	ATOM	1925	CG	LEU	61B	-53.412	54.866	-70.262	1.00	2.10	C
	ATOM	1926	CD1	LEU	61B	-52.891	56.015	-69.410	1.00	3.60	C
	ATOM	1927	CD2	LEU	61B	-53.857	53.697	-69.387	1.00	5.37	C
40	ATOM	1928	C	LEU	61B	-52.990	55.853	-72.928	1.00	1.03	C
	ATOM	1929	O	LEU	61B	-52.961	54.875	-73.677	1.00	2.58	O
	ATOM	1930	N	ILE	62B	-51.921	56.591	-72.648	1.00	1.00	N
	ATOM	1931	CA	ILE	62B	-50.605	56.219	-73.134	1.00	1.60	C
	ATOM	1932	CB	ILE	62B	-49.993	57.253	-74.094	1.00	1.65	C
45	ATOM	1933	CG2	ILE	62B	-48.575	56.830	-74.463	1.00	2.13	C
	ATOM	1934	CG1	ILE	62B	-50.833	57.344	-75.368	1.00	4.34	C
	ATOM	1935	CD1	ILE	62B	-50.240	58.272	-76.411	1.00	4.19	C
	ATOM	1936	C	ILE	62B	-49.756	56.149	-71.882	1.00	1.00	C
	ATOM	1937	O	ILE	62B	-49.363	57.180	-71.331	1.00	2.27	O
50	ATOM	1938	N	PRO	63B	-49.500	54.930	-71.383	1.00	1.00	N
	ATOM	1939	CD	PRO	63B	-49.978	53.624	-71.865	1.00	2.24	C
	ATOM	1940	CA	PRO	63B	-48.682	54.775	-70.176	1.00	1.00	C
	ATOM	1941	CB	PRO	63B	-48.591	53.259	-70.015	1.00	2.21	C
	ATOM	1942	CG	PRO	63B	-49.872	52.771	-70.617	1.00	1.00	C
55	ATOM	1943	C	PRO	63B	-47.306	55.394	-70.402	1.00	1.74	C
	ATOM	1944	O	PRO	63B	-46.777	55.329	-71.511	1.00	1.00	O
	ATOM	1945	N	THR	64B	-46.730	55.983	-69.355	1.00	1.00	N
	ATOM	1946	CA	THR	64B	-45.403	56.583	-69.450	1.00	2.14	C
	ATOM	1947	CB	THR	64B	-45.422	58.085	-69.102	1.00	3.01	C
60	ATOM	1948	OG1	THR	64B	-45.980	58.271	-67.794	1.00	4.59	O
	ATOM	1949	CG2	THR	64B	-46.246	58.849	-70.132	1.00	2.35	C

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	ATOM	1950	C	THR	64B	-44.411	55.861	-68.543	1.00	1.68 C
	ATOM	1951	O	THR	64B	-43.310	56.348	-68.285	1.00	2.15 O
	ATOM	1952	N	THR	65B	-44.830	54.702	-68.045	1.00	2.52 N
	ATOM	1953	CA	THR	65B	-43.968	53.869	-67.230	1.00	3.10 C
5	ATOM	1954	CB	THR	65B	-44.771	52.818	-66.447	1.00	4.88 C
	ATOM	1955	OG1	THR	65B	-45.573	52.064	-67.367	1.00	6.05 O
	ATOM	1956	CG2	THR	65B	-45.674	53.475	-65.422	1.00	3.38 C
	ATOM	1957	C	THR	65B	-43.227	53.139	-68.335	1.00	4.97 C
	ATOM	1958	O	THR	65B	-43.569	53.289	-69.509	1.00	6.98 O
10	ATOM	1959	N	TYR	66B	-42.216	52.356	-68.002	1.00	8.08 N
	ATOM	1960	CA	TYR	66B	-41.566	51.621	-69.072	1.00	9.06 C
	ATOM	1961	CB	TYR	66B	-40.254	51.004	-68.599	1.00	11.12 C
	ATOM	1962	CG	TYR	66B	-39.097	51.919	-68.916	1.00	12.07 C
	ATOM	1963	CD1	TYR	66B	-38.364	52.533	-67.906	1.00	11.75 C
15	ATOM	1964	CE1	TYR	66B	-37.360	53.449	-68.210	1.00	13.37 C
	ATOM	1965	CD2	TYR	66B	-38.795	52.236	-70.241	1.00	14.31 C
	ATOM	1966	CE2	TYR	66B	-37.797	53.150	-70.557	1.00	14.45 C
	ATOM	1967	CZ	TYR	66B	-37.087	53.753	-69.540	1.00	15.31 C
	ATOM	1968	OH	TYR	66B	-36.116	54.669	-69.859	1.00	15.24 O
20	ATOM	1969	C	TYR	66B	-42.561	50.560	-69.518	1.00	7.59 C
	ATOM	1970	O	TYR	66B	-43.475	50.225	-68.775	1.00	8.33 O
	ATOM	1971	N	MET	67B	-42.400	50.060	-70.737	1.00	7.28 N
	ATOM	1972	CA	MET	67B	-43.310	49.057	-71.278	1.00	8.78 C
	ATOM	1973	CB	MET	67B	-42.746	48.493	-72.589	1.00	7.74 C
25	ATOM	1974	CG	MET	67B	-43.680	47.533	-73.320	1.00	6.74 C
	ATOM	1975	SD	MET	67B	-45.178	48.322	-73.938	1.00	11.73 S
	ATOM	1976	CE	MET	67B	-44.710	48.658	-75.652	1.00	9.90 C
	ATOM	1977	C	MET	67B	-43.615	47.901	-70.323	1.00	8.91 C
	ATOM	1978	O	MET	67B	-44.739	47.411	-70.293	1.00	7.86 O
30	ATOM	1979	N	ASN	68B	-42.626	47.469	-69.541	1.00	10.61 N
	ATOM	1980	CA	ASN	68B	-42.830	46.340	-68.626	1.00	13.25 C
	ATOM	1981	CB	ASN	68B	-41.488	45.715	-68.241	1.00	19.28 C
	ATOM	1982	CG	ASN	68B	-40.976	44.755	-69.292	1.00	23.82 C
	ATOM	1983	OD1	ASN	68B	-40.569	45.167	-70.381	1.00	27.78 O
35	ATOM	1984	ND2	ASN	68B	-41.009	43.458	-68.978	1.00	25.61 N
	ATOM	1985	C	ASN	68B	-43.644	46.596	-67.360	1.00	11.35 C
	ATOM	1986	O	ASN	68B	-43.930	45.662	-66.609	1.00	8.74 O
	ATOM	1987	N	ARG	69B	-44.017	47.849	-67.123	1.00	9.82 N
	ATOM	1988	CA	ARG	69B	-44.818	48.195	-65.956	1.00	8.98 C
40	ATOM	1989	CB	ARG	69B	-44.010	49.060	-64.989	1.00	10.02 C
	ATOM	1990	CG	ARG	69B	-42.903	48.317	-64.265	1.00	11.52 C
	ATOM	1991	CD	ARG	69B	-43.470	47.386	-63.206	1.00	11.69 C
	ATOM	1992	NE	ARG	69B	-42.418	46.630	-62.537	1.00	15.30 N
	ATOM	1993	CZ	ARG	69B	-41.741	45.641	-63.106	1.00	14.17 C
45	ATOM	1994	NH1	ARG	69B	-40.793	45.006	-62.428	1.00	16.63 N
	ATOM	1995	NH2	ARG	69B	-42.026	45.280	-64.348	1.00	14.44 N
	ATOM	1996	C	ARG	69B	-46.066	48.954	-66.388	1.00	6.66 C
	ATOM	1997	O	ARG	69B	-46.715	49.604	-65.575	1.00	5.49 O
	ATOM	1998	N	SER	70B	-46.408	48.859	-67.669	1.00	6.01 N
50	ATOM	1999	CA	SER	70B	-47.573	49.574	-68.180	1.00	4.00 C
	ATOM	2000	CB	SER	70B	-47.634	49.468	-69.709	1.00	4.50 C
	ATOM	2001	OG	SER	70B	-47.607	48.129	-70.154	1.00	8.31 O
	ATOM	2002	C	SER	70B	-48.884	49.116	-67.545	1.00	3.40 C
	ATOM	2003	O	SER	70B	-49.890	49.828	-67.590	1.00	4.53 O
55	ATOM	2004	N	GLY	71B	-48.864	47.936	-66.936	1.00	3.05 N
	ATOM	2005	CA	GLY	71B	-50.049	47.418	-66.280	1.00	3.14 C
	ATOM	2006	C	GLY	71B	-50.446	48.255	-65.079	1.00	2.33 C
	ATOM	2007	O	GLY	71B	-51.625	48.327	-64.734	1.00	4.33 O
	ATOM	2008	N	GLN	72B	-49.470	48.882	-64.425	1.00	1.56 N
60	ATOM	2009	CA	GLN	72B	-49.778	49.724	-63.276	1.00	4.39 C
	ATOM	2010	CB	GLN	72B	-48.493	50.149	-62.556	1.00	7.88 C

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	ATOM	2011	CG	GLN	72B	-47.971	49.091	-61.604	1.00	13.35	C
	ATOM	2012	CD	GLN	72B	-46.643	49.454	-60.970	1.00	18.57	C
	ATOM	2013	OE1	GLN	72B	-46.510	50.496	-60.323	1.00	22.32	O
	ATOM	2014	NE2	GLN	72B	-45.650	48.587	-61.145	1.00	19.02	N
5	ATOM	2015	C	GLN	72B	-50.550	50.953	-63.745	1.00	2.94	C
	ATOM	2016	O	GLN	72B	-51.397	51.478	-63.032	1.00	2.33	O
	ATOM	2017	N	SER	73B	-50.245	51.405	-64.955	1.00	3.74	N
	ATOM	2018	CA	SER	73B	-50.916	52.558	-65.531	1.00	2.74	C
10	ATOM	2019	CB	SER	73B	-50.174	53.014	-66.796	1.00	3.50	C
	ATOM	2020	OG	SER	73B	-50.846	54.095	-67.422	1.00	2.01	O
	ATOM	2021	C	SER	73B	-52.364	52.197	-65.871	1.00	2.96	C
	ATOM	2022	O	SER	73B	-53.309	52.861	-65.435	1.00	1.49	O
	ATOM	2023	N	VAL	74B	-52.538	51.123	-66.632	1.00	2.23	N
	ATOM	2024	CA	VAL	74B	-53.873	50.702	-67.029	1.00	2.54	C
15	ATOM	2025	CB	VAL	74B	-53.805	49.459	-67.951	1.00	3.62	C
	ATOM	2026	CG1	VAL	74B	-53.000	49.790	-69.219	1.00	1.16	C
	ATOM	2027	CG2	VAL	74B	-53.183	48.295	-67.217	1.00	9.12	C
	ATOM	2028	C	VAL	74B	-54.779	50.407	-65.830	1.00	1.17	C
20	ATOM	2029	O	VAL	74B	-55.935	50.845	-65.790	1.00	1.00	O
	ATOM	2030	N	ALA	75B	-54.249	49.678	-64.851	1.00	1.98	N
	ATOM	2031	CA	ALA	75B	-55.007	49.319	-63.654	1.00	2.83	C
	ATOM	2032	CB	ALA	75B	-54.200	48.334	-62.804	1.00	3.98	C
	ATOM	2033	C	ALA	75B	-55.365	50.558	-62.829	1.00	1.44	C
	ATOM	2034	O	ALA	75B	-56.444	50.639	-62.238	1.00	1.56	O
25	ATOM	2035	N	ALA	76B	-54.465	51.532	-62.792	1.00	3.27	N
	ATOM	2036	CA	ALA	76B	-54.720	52.745	-62.034	1.00	1.28	C
	ATOM	2037	CB	ALA	76B	-53.464	53.635	-62.023	1.00	1.00	C
	ATOM	2038	C	ALA	76B	-55.895	53.520	-62.613	1.00	3.77	C
	ATOM	2039	O	ALA	76B	-56.739	54.033	-61.873	1.00	1.48	O
30	ATOM	2040	N	LEU	77B	-55.946	53.615	-63.937	1.00	3.04	N
	ATOM	2041	CA	LEU	77B	-57.019	54.361	-64.583	1.00	3.49	C
	ATOM	2042	CB	LEU	77B	-56.587	54.797	-65.985	1.00	4.49	C
	ATOM	2043	CG	LEU	77B	-57.535	55.725	-66.759	1.00	4.68	C
	ATOM	2044	CD1	LEU	77B	-57.720	57.050	-66.029	1.00	7.73	C
35	ATOM	2045	CD2	LEU	77B	-56.954	55.961	-68.139	1.00	6.25	C
	ATOM	2046	C	LEU	77B	-58.307	53.544	-64.656	1.00	2.77	C
	ATOM	2047	O	LEU	77B	-59.385	54.049	-64.362	1.00	2.66	O
	ATOM	2048	N	ALA	78B	-58.190	52.279	-65.042	1.00	4.00	N
	ATOM	2049	CA	ALA	78B	-59.366	51.420	-65.147	1.00	5.10	C
40	ATOM	2050	CB	ALA	78B	-58.968	50.067	-65.708	1.00	5.23	C
	ATOM	2051	C	ALA	78B	-60.021	51.254	-63.778	1.00	6.08	C
	ATOM	2052	O	ALA	78B	-61.247	51.330	-63.645	1.00	6.49	O
	ATOM	2053	N	GLY	79B	-59.193	51.045	-62.761	1.00	5.42	N
	ATOM	2054	CA	GLY	79B	-59.711	50.865	-61.420	1.00	4.24	C
45	ATOM	2055	C	GLY	79B	-60.444	52.085	-60.916	1.00	5.54	C
	ATOM	2056	O	GLY	79B	-61.544	51.983	-60.365	1.00	3.87	O
	ATOM	2057	N	PHE	80B	-59.829	53.246	-61.114	1.00	4.36	N
	ATOM	2058	CA	PHE	80B	-60.385	54.515	-60.673	1.00	5.56	C
	ATOM	2059	CB	PHE	80B	-59.478	55.655	-61.124	1.00	5.80	C
50	ATOM	2060	CG	PHE	80B	-59.785	56.960	-60.463	1.00	2.41	C
	ATOM	2061	CD1	PHE	80B	-59.175	57.299	-59.261	1.00	2.37	C
	ATOM	2062	CD2	PHE	80B	-60.703	57.841	-61.025	1.00	1.22	C
	ATOM	2063	CE1	PHE	80B	-59.475	58.505	-58.621	1.00	3.66	C
	ATOM	2064	CE2	PHE	80B	-61.012	59.047	-60.394	1.00	4.14	C
55	ATOM	2065	CZ	PHE	80B	-60.398	59.379	-59.191	1.00	3.11	C
	ATOM	2066	C	PHE	80B	-61.798	54.772	-61.191	1.00	5.32	C
	ATOM	2067	O	PHE	80B	-62.659	55.280	-60.462	1.00	5.33	O
	ATOM	2068	N	PHE	81B	-62.031	54.428	-62.453	1.00	5.93	N
	ATOM	2069	CA	PHE	81B	-63.340	54.641	-63.068	1.00	6.93	C
60	ATOM	2070	CB	PHE	81B	-63.159	55.287	-64.442	1.00	8.01	C
	ATOM	2071	CG	PHE	81B	-62.683	56.706	-64.372	1.00	8.00	C

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	ATOM	2072	CD1	PHE	81B	-63.537	57.712	-63.921	1.00	10.51	C
	ATOM	2073	CD2	PHE	81B	-61.370	57.037	-64.698	1.00	9.92	C
	ATOM	2074	CE1	PHE	81B	-63.091	59.022	-63.793	1.00	8.70	C
5	ATOM	2075	CE2	PHE	81B	-60.912	58.350	-64.572	1.00	9.39	C
	ATOM	2076	CZ	PHE	81B	-61.774	59.343	-64.117	1.00	9.39	C
	ATOM	2077	C	PHE	81B	-64.171	53.374	-63.187	1.00	8.16	C
	ATOM	2078	O	PHE	81B	-65.185	53.353	-63.891	1.00	9.04	O
	ATOM	2079	N	ARG	82B	-63.743	52.329	-62.483	1.00	8.06	N
10	ATOM	2080	CA	ARG	82B	-64.427	51.036	-62.485	1.00	9.95	C
	ATOM	2081	CB	ARG	82B	-65.736	51.117	-61.689	1.00	13.35	C
	ATOM	2082	CG	ARG	82B	-65.562	51.440	-60.207	1.00	19.57	C
	ATOM	2083	CD	ARG	82B	-65.327	52.925	-59.981	1.00	25.43	C
	ATOM	2084	NE	ARG	82B	-65.255	53.268	-58.562	1.00	30.97	N
15	ATOM	2085	CZ	ARG	82B	-64.224	52.991	-57.773	1.00	32.28	C
	ATOM	2086	NH1	ARG	82B	-64.254	53.339	-56.495	1.00	33.29	N
	ATOM	2087	NH2	ARG	82B	-63.157	52.375	-58.262	1.00	34.75	N
	ATOM	2088	C	ARG	82B	-64.716	50.520	-63.893	1.00	8.99	C
	ATOM	2089	O	ARG	82B	-65.856	50.175	-64.221	1.00	8.59	O
	ATOM	2090	N	ILE	83B	-63.674	50.468	-64.718	1.00	6.16	N
20	ATOM	2091	CA	ILE	83B	-63.787	49.992	-66.090	1.00	5.00	C
	ATOM	2092	CB	ILE	83B	-62.909	50.842	-67.050	1.00	3.62	C
	ATOM	2093	CG2	ILE	83B	-62.964	50.265	-68.469	1.00	3.34	C
	ATOM	2094	CG1	ILE	83B	-63.384	52.301	-67.034	1.00	4.33	C
	ATOM	2095	CD1	ILE	83B	-62.498	53.252	-67.829	1.00	5.71	C
25	ATOM	2096	C	ILE	83B	-63.353	48.525	-66.167	1.00	4.95	C
	ATOM	2097	O	ILE	83B	-62.212	48.181	-65.852	1.00	5.39	O
	ATOM	2098	N	ALA	84B	-64.279	47.667	-66.584	1.00	5.42	N
	ATOM	2099	CA	ALA	84B	-64.019	46.235	-66.705	1.00	4.82	C
	ATOM	2100	CB	ALA	84B	-65.338	45.488	-66.802	1.00	4.52	C
30	ATOM	2101	C	ALA	84B	-63.145	45.898	-67.913	1.00	5.16	C
	ATOM	2102	O	ALA	84B	-63.172	46.591	-68.925	1.00	4.39	O
	ATOM	2103	N	PRO	85B	-62.370	44.806	-67.824	1.00	5.84	N
	ATOM	2104	CD	PRO	85B	-62.169	43.931	-66.656	1.00	4.89	C
	ATOM	2105	CA	PRO	85B	-61.497	44.404	-68.930	1.00	5.59	C
35	ATOM	2106	CB	PRO	85B	-60.976	43.045	-68.475	1.00	6.29	C
	ATOM	2107	CG	PRO	85B	-60.868	43.234	-66.995	1.00	7.89	C
	ATOM	2108	C	PRO	85B	-62.217	44.339	-70.272	1.00	5.85	C
	ATOM	2109	O	PRO	85B	-61.647	44.705	-71.300	1.00	5.60	O
	ATOM	2110	N	ASP	86B	-63.473	43.900	-70.259	1.00	3.97	N
40	ATOM	2111	CA	ASP	86B	-64.250	43.798	-71.492	1.00	6.06	C
	ATOM	2112	CB	ASP	86B	-65.624	43.182	-71.206	1.00	6.85	C
	ATOM	2113	CG	ASP	86B	-66.303	42.666	-72.461	1.00	10.85	C
	ATOM	2114	OD1	ASP	86B	-65.620	42.009	-73.275	1.00	12.25	O
	ATOM	2115	OD2	ASP	86B	-67.521	42.898	-72.632	1.00	11.09	O
45	ATOM	2116	C	ASP	86B	-64.433	45.145	-72.189	1.00	3.21	C
	ATOM	2117	O	ASP	86B	-64.681	45.195	-73.385	1.00	3.89	O
	ATOM	2118	N	ALA	87B	-64.303	46.236	-71.443	1.00	3.69	N
	ATOM	2119	CA	ALA	87B	-64.468	47.569	-72.015	1.00	2.12	C
	ATOM	2120	CB	ALA	87B	-65.267	48.449	-71.055	1.00	2.35	C
50	ATOM	2121	C	ALA	87B	-63.141	48.246	-72.360	1.00	2.57	C
	ATOM	2122	O	ALA	87B	-63.100	49.447	-72.616	1.00	3.65	O
	ATOM	2123	N	ILE	88B	-62.065	47.473	-72.368	1.00	3.04	N
	ATOM	2124	CA	ILE	88B	-60.746	48.006	-72.679	1.00	2.52	C
	ATOM	2125	CB	ILE	88B	-59.727	47.650	-71.568	1.00	2.50	C
55	ATOM	2126	CG2	ILE	88B	-58.341	48.182	-71.945	1.00	1.08	C
	ATOM	2127	CG1	ILE	88B	-60.203	48.207	-70.223	1.00	3.69	C
	ATOM	2128	CD1	ILE	88B	-59.321	47.810	-69.035	1.00	2.16	C
	ATOM	2129	C	ILE	88B	-60.199	47.482	-74.007	1.00	1.45	C
	ATOM	2130	O	ILE	88B	-60.313	46.304	-74.315	1.00	3.14	O
60	ATOM	2131	N	LEU	89B	-59.608	48.376	-74.789	1.00	3.23	N
	ATOM	2132	CA	LEU	89B	-58.989	48.024	-76.066	1.00	1.44	C

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	ATOM	2133	CB	LEU	89B	-59.652	48.784	-77.219	1.00	3.11	C
	ATOM	2134	CG	LEU	89B	-58.905	48.749	-78.562	1.00	2.58	C
	ATOM	2135	CD1	LEU	89B	-58.770	47.305	-79.051	1.00	1.00	C
	ATOM	2136	CD2	LEU	89B	-59.660	49.606	-79.587	1.00	1.46	C
5	ATOM	2137	C	LEU	89B	-57.523	48.441	-75.962	1.00	2.19	C
	ATOM	2138	O	LEU	89B	-57.235	49.593	-75.652	1.00	2.78	O
	ATOM	2139	N	VAL	90B	-56.600	47.513	-76.204	1.00	1.54	N
	ATOM	2140	CA	VAL	90B	-55.180	47.839	-76.124	1.00	1.83	C
	ATOM	2141	CB	VAL	90B	-54.452	46.929	-75.107	1.00	4.14	C
	ATOM	2142	CG1	VAL	90B	-52.972	47.306	-75.039	1.00	1.73	C
10	ATOM	2143	CG2	VAL	90B	-55.095	47.057	-73.731	1.00	2.19	C
	ATOM	2144	C	VAL	90B	-54.472	47.727	-77.476	1.00	2.14	C
	ATOM	2145	O	VAL	90B	-54.399	46.645	-78.062	1.00	1.00	O
	ATOM	2146	N	ALA	91B	-53.953	48.849	-77.970	1.00	2.74	N
	ATOM	2147	CA	ALA	91B	-53.232	48.864	-79.241	1.00	2.45	C
	ATOM	2148	CB	ALA	91B	-53.389	50.221	-79.932	1.00	1.00	C
15	ATOM	2149	C	ALA	91B	-51.758	48.565	-78.976	1.00	3.66	C
	ATOM	2150	O	ALA	91B	-51.150	49.116	-78.058	1.00	2.32	O
	ATOM	2151	N	HIS	92B	-51.178	47.695	-79.792	1.00	2.68	N
	ATOM	2152	CA	HIS	92B	-49.795	47.318	-79.588	1.00	5.01	C
	ATOM	2153	CB	HIS	92B	-49.725	46.243	-78.493	1.00	3.10	C
	ATOM	2154	CG	HIS	92B	-50.197	44.893	-78.939	1.00	2.03	C
20	ATOM	2155	CD2	HIS	92B	-49.538	43.881	-79.548	1.00	1.00	C
	ATOM	2156	ND1	HIS	92B	-51.504	44.473	-78.805	1.00	4.66	N
	ATOM	2157	CE1	HIS	92B	-51.628	43.257	-79.308	1.00	1.00	C
	ATOM	2158	NE2	HIS	92B	-50.448	42.875	-79.766	1.00	1.08	N
	ATOM	2159	C	HIS	92B	-49.149	46.797	-80.866	1.00	3.68	C
	ATOM	2160	O	HIS	92B	-49.842	46.384	-81.798	1.00	3.85	O
25	ATOM	2161	N	ASP	93B	-47.820	46.826	-80.902	1.00	4.00	N
	ATOM	2162	CA	ASP	93B	-47.067	46.334	-82.050	1.00	1.29	C
	ATOM	2163	CB	ASP	93B	-45.605	46.785	-81.960	1.00	2.66	C
	ATOM	2164	CG	ASP	93B	-44.962	46.446	-80.621	1.00	1.00	C
	ATOM	2165	OD1	ASP	93B	-43.926	45.745	-80.607	1.00	3.29	O
	ATOM	2166	OD2	ASP	93B	-45.485	46.888	-79.582	1.00	2.95	O
30	ATOM	2167	C	ASP	93B	-47.159	44.812	-82.096	1.00	2.33	C
	ATOM	2168	O	ASP	93B	-47.123	44.147	-81.060	1.00	2.27	O
	ATOM	2169	N	GLU	94B	-47.301	44.266	-83.299	1.00	2.49	N
	ATOM	2170	CA	GLU	94B	-47.419	42.822	-83.479	1.00	1.67	C
	ATOM	2171	CB	GLU	94B	-48.801	42.482	-84.040	1.00	1.00	C
	ATOM	2172	CG	GLU	94B	-49.007	40.996	-84.320	1.00	1.84	C
35	ATOM	2173	CD	GLU	94B	-48.836	40.156	-83.072	1.00	4.62	C
	ATOM	2174	OE1	GLU	94B	-49.629	40.323	-82.123	1.00	3.08	O
	ATOM	2175	OE2	GLU	94B	-47.897	39.336	-83.039	1.00	9.18	O
	ATOM	2176	C	GLU	94B	-46.342	42.268	-84.409	1.00	1.13	C
	ATOM	2177	O	GLU	94B	-46.314	42.582	-85.598	1.00	1.00	O
	ATOM	2178	N	LEU	95B	-45.481	41.418	-83.861	1.00	1.67	N
40	ATOM	2179	CA	LEU	95B	-44.387	40.813	-84.619	1.00	1.00	C
	ATOM	2180	CB	LEU	95B	-43.424	40.096	-83.657	1.00	2.42	C
	ATOM	2181	CG	LEU	95B	-42.610	40.972	-82.688	1.00	1.60	C
	ATOM	2182	CD1	LEU	95B	-42.106	40.143	-81.518	1.00	1.61	C
	ATOM	2183	CD2	LEU	95B	-41.456	41.619	-83.428	1.00	2.70	C
	ATOM	2184	C	LEU	95B	-44.850	39.816	-85.680	1.00	2.71	C
45	ATOM	2185	O	LEU	95B	-44.208	39.664	-86.723	1.00	1.95	O
	ATOM	2186	N	ASP	96B	-45.968	39.148	-85.423	1.00	3.16	N
	ATOM	2187	CA	ASP	96B	-46.460	38.125	-86.337	1.00	2.26	C
	ATOM	2188	CB	ASP	96B	-47.302	37.108	-85.568	1.00	4.92	C
	ATOM	2189	CG	ASP	96B	-46.463	36.239	-84.650	1.00	4.83	C
	ATOM	2190	OD1	ASP	96B	-45.223	36.197	-84.824	1.00	6.60	O
50	ATOM	2191	OD2	ASP	96B	-47.048	35.583	-83.763	1.00	8.23	O
	ATOM	2192	C	ASP	96B	-47.209	38.575	-87.579	1.00	4.06	C
	ATOM	2193	O	ASP	96B	-47.672	37.741	-88.359	1.00	1.38	O

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	ATOM	2194	N	MET	97B	-47.324	39.887	-87.758	1.00	1.92	N
	ATOM	2195	CA	MET	97B	-47.982	40.464	-88.922	1.00	4.52	C
	ATOM	2196	CB	MET	97B	-49.296	41.126	-88.521	1.00	6.05	C
	ATOM	2197	CG	MET	97B	-50.325	40.148	-88.014	1.00	5.91	C
5	ATOM	2198	SD	MET	97B	-51.790	40.977	-87.382	1.00	10.42	S
	ATOM	2199	CE	MET	97B	-52.329	39.813	-86.176	1.00	6.99	C
	ATOM	2200	C	MET	97B	-47.033	41.495	-89.514	1.00	4.54	C
	ATOM	2201	O	MET	97B	-46.381	42.234	-88.785	1.00	2.81	O
	ATOM	2202	N	PRO	98B	-46.933	41.544	-90.847	1.00	4.20	N
10	ATOM	2203	CD	PRO	98B	-47.684	40.737	-91.831	1.00	6.83	C
	ATOM	2204	CA	PRO	98B	-46.048	42.496	-91.520	1.00	5.03	C
	ATOM	2205	CB	PRO	98B	-46.024	41.974	-92.949	1.00	5.36	C
	ATOM	2206	CG	PRO	98B	-47.440	41.492	-93.126	1.00	6.31	C
	ATOM	2207	C	PRO	98B	-46.559	43.929	-91.462	1.00	3.83	C
15	ATOM	2208	O	PRO	98B	-47.741	44.169	-91.212	1.00	4.97	O
	ATOM	2209	N	PRO	99B	-45.666	44.904	-91.679	1.00	4.29	N
	ATOM	2210	CD	PRO	99B	-44.201	44.809	-91.774	1.00	1.78	C
	ATOM	2211	CA	PRO	99B	-46.102	46.301	-91.651	1.00	3.53	C
	ATOM	2212	CB	PRO	99B	-44.835	47.058	-92.036	1.00	3.55	C
20	ATOM	2213	CG	PRO	99B	-43.763	46.228	-91.427	1.00	2.70	C
	ATOM	2214	C	PRO	99B	-47.212	46.463	-92.692	1.00	4.96	C
	ATOM	2215	O	PRO	99B	-47.126	45.888	-93.774	1.00	5.42	O
	ATOM	2216	N	GLY	100B	-48.255	47.222	-92.362	1.00	6.04	N
	ATOM	2217	CA	GLY	100B	-49.345	47.416	-93.304	1.00	4.44	C
25	ATOM	2218	C	GLY	100B	-50.570	46.577	-92.986	1.00	6.31	C
	ATOM	2219	O	GLY	100B	-51.618	46.704	-93.622	1.00	4.97	O
	ATOM	2220	N	VAL	101B	-50.446	45.715	-91.988	1.00	5.55	N
	ATOM	2221	CA	VAL	101B	-51.553	44.856	-91.599	1.00	8.08	C
	ATOM	2222	CB	VAL	101B	-51.201	43.370	-91.837	1.00	8.84	C
30	ATOM	2223	CG1	VAL	101B	-52.310	42.465	-91.306	1.00	8.12	C
	ATOM	2224	CG2	VAL	101B	-50.993	43.135	-93.325	1.00	7.19	C
	ATOM	2225	C	VAL	101B	-51.940	45.060	-90.140	1.00	10.30	C
	ATOM	2226	O	VAL	101B	-51.079	45.098	-89.256	1.00	10.96	O
	ATOM	2227	N	ALA	102B	-53.244	45.194	-89.904	1.00	9.40	N
35	ATOM	2228	CA	ALA	102B	-53.772	45.390	-88.564	1.00	8.25	C
	ATOM	2229	CB	ALA	102B	-54.176	46.852	-88.362	1.00	8.84	C
	ATOM	2230	C	ALA	102B	-54.972	44.484	-88.335	1.00	7.82	C
	ATOM	2231	O	ALA	102B	-55.860	44.381	-89.182	1.00	6.14	O
	ATOM	2232	N	LYS	103B	-54.990	43.820	-87.187	1.00	7.51	N
40	ATOM	2233	CA	LYS	103B	-56.095	42.940	-86.854	1.00	8.21	C
	ATOM	2234	CB	LYS	103B	-55.711	41.480	-87.112	1.00	11.34	C
	ATOM	2235	CG	LYS	103B	-55.466	41.168	-88.577	1.00	14.53	C
	ATOM	2236	CD	LYS	103B	-55.196	39.687	-88.805	1.00	18.62	C
	ATOM	2237	CE	LYS	103B	-54.758	39.424	-90.243	1.00	20.77	C
45	ATOM	2238	NZ	LYS	103B	-55.747	39.935	-91.238	1.00	25.06	N
	ATOM	2239	C	LYS	103B	-56.517	43.133	-85.404	1.00	7.70	C
	ATOM	2240	O	LYS	103B	-55.767	43.678	-84.590	1.00	5.76	O
	ATOM	2241	N	LEU	104B	-57.730	42.691	-85.095	1.00	6.01	N
	ATOM	2242	CA	LEU	104B	-58.282	42.809	-83.753	1.00	5.64	C
50	ATOM	2243	CB	LEU	104B	-59.658	43.478	-83.810	1.00	4.96	C
	ATOM	2244	CG	LEU	104B	-59.657	44.971	-84.135	1.00	5.33	C
	ATOM	2245	CD1	LEU	104B	-61.024	45.424	-84.615	1.00	3.79	C
	ATOM	2246	CD2	LEU	104B	-59.242	45.726	-82.900	1.00	3.42	C
	ATOM	2247	C	LEU	104B	-58.408	41.428	-83.130	1.00	5.65	C
55	ATOM	2248	O	LEU	104B	-58.615	40.439	-83.832	1.00	5.29	O
	ATOM	2249	N	LYS	105B	-58.282	41.364	-81.811	1.00	5.44	N
	ATOM	2250	CA	LYS	105B	-58.384	40.091	-81.113	1.00	9.90	C
	ATOM	2251	CB	LYS	105B	-57.018	39.400	-81.069	1.00	9.41	C
	ATOM	2252	CG	LYS	105B	-57.028	38.090	-80.307	1.00	13.46	C
60	ATOM	2253	CD	LYS	105B	-55.673	37.420	-80.315	1.00	17.56	C
	ATOM	2254	CE	LYS	105B	-55.770	36.030	-79.691	1.00	19.05	C

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	ATOM	2255	NZ	LYS	105B	-54.451	35.356	-79.613	1.00	21.08	N
	ATOM	2256	C	LYS	105B	-58.897	40.276	-79.696	1.00	10.24	C
	ATOM	2257	O	LYS	105B	-58.593	41.268	-79.040	1.00	8.95	O
5	ATOM	2258	N	THR	106B	-59.675	39.310	-79.226	1.00	12.32	N
	ATOM	2259	CA	THR	106B	-60.206	39.369	-77.878	1.00	15.54	C
	ATOM	2260	CB	THR	106B	-61.746	39.250	-77.883	1.00	19.09	C
	ATOM	2261	OG1	THR	106B	-62.222	39.131	-76.536	1.00	22.18	O
	ATOM	2262	CG2	THR	106B	-62.189	38.043	-78.695	1.00	21.39	C
10	ATOM	2263	C	THR	106B	-59.587	38.242	-77.053	1.00	15.37	C
	ATOM	2264	O	THR	106B	-59.593	37.081	-77.465	1.00	13.68	O
	ATOM	2265	N	GLY	107B	-59.026	38.599	-75.903	1.00	14.38	N
	ATOM	2266	CA	GLY	107B	-58.416	37.608	-75.039	1.00	15.07	C
	ATOM	2267	C	GLY	107B	-57.126	37.032	-75.587	1.00	15.75	C
15	ATOM	2268	O	GLY	107B	-56.468	37.640	-76.432	1.00	14.52	O
	ATOM	2269	N	GLY	108B	-56.766	35.848	-75.106	1.00	16.62	N
	ATOM	2270	CA	GLY	108B	-55.542	35.210	-75.557	1.00	18.82	C
	ATOM	2271	C	GLY	108B	-54.374	35.569	-74.658	1.00	19.50	C
	ATOM	2272	O	GLY	108B	-54.553	36.256	-73.652	1.00	21.15	O
20	ATOM	2273	N	GLY	109B	-53.180	35.109	-75.021	1.00	18.99	N
	ATOM	2274	CA	GLY	109B	-51.997	35.393	-74.225	1.00	18.51	C
	ATOM	2275	C	GLY	109B	-51.373	36.738	-74.546	1.00	17.94	C
	ATOM	2276	O	GLY	109B	-52.056	37.649	-75.006	1.00	17.28	O
	ATOM	2277	N	HIS	110B	-50.073	36.866	-74.300	1.00	17.07	N
25	ATOM	2278	CA	HIS	110B	-49.371	38.110	-74.572	1.00	16.68	C
	ATOM	2279	CB	HIS	110B	-48.585	38.561	-73.338	1.00	15.45	C
	ATOM	2280	CG	HIS	110B	-47.773	37.474	-72.709	1.00	13.99	C
	ATOM	2281	CD2	HIS	110B	-46.544	36.992	-73.009	1.00	12.22	C
	ATOM	2282	ND1	HIS	110B	-48.226	36.730	-71.642	1.00	14.67	N
30	ATOM	2283	CE1	HIS	110B	-47.310	35.837	-71.311	1.00	12.51	C
	ATOM	2284	NE2	HIS	110B	-46.280	35.975	-72.125	1.00	13.04	N
	ATOM	2285	C	HIS	110B	-48.421	37.976	-75.754	1.00	17.20	C
	ATOM	2286	O	HIS	110B	-47.856	38.967	-76.219	1.00	15.23	O
	ATOM	2287	N	GLY	111B	-48.247	36.748	-76.235	1.00	17.56	N
35	ATOM	2288	CA	GLY	111B	-47.355	36.518	-77.359	1.00	18.79	C
	ATOM	2289	C	GLY	111B	-45.929	36.943	-77.066	1.00	18.68	C
	ATOM	2290	O	GLY	111B	-45.158	37.243	-77.980	1.00	19.46	O
	ATOM	2291	N	GLY	112B	-45.575	36.965	-75.785	1.00	18.29	N
	ATOM	2292	CA	GLY	112B	-44.237	37.362	-75.391	1.00	16.30	C
40	ATOM	2293	C	GLY	112B	-44.087	38.870	-75.298	1.00	15.47	C
	ATOM	2294	O	GLY	112B	-43.011	39.374	-74.995	1.00	16.30	O
	ATOM	2295	N	HIS	113B	-45.170	39.598	-75.555	1.00	13.77	N
	ATOM	2296	CA	HIS	113B	-45.124	41.052	-75.493	1.00	11.72	C
	ATOM	2297	CB	HIS	113B	-46.330	41.649	-76.227	1.00	9.23	C
45	ATOM	2298	CG	HIS	113B	-46.215	43.118	-76.482	1.00	5.60	C
	ATOM	2299	CD2	HIS	113B	-45.995	43.811	-77.625	1.00	5.15	N
	ATOM	2300	ND1	HIS	113B	-46.327	44.058	-75.481	1.00	4.67	C
	ATOM	2301	CE1	HIS	113B	-46.186	45.266	-75.996	1.00	5.20	N
	ATOM	2302	NE2	HIS	113B	-45.983	45.144	-77.295	1.00	11.35	C
50	ATOM	2303	C	HIS	113B	-45.105	41.499	-74.031	1.00	9.97	O
	ATOM	2304	O	HIS	113B	-46.061	41.283	-73.291	1.00	11.65	N
	ATOM	2305	N	ASN	114B	-43.992	42.107	-73.635	1.00	13.51	C
	ATOM	2306	CA	ASN	114B	-43.777	42.594	-72.277	1.00	20.27	C
	ATOM	2307	CB	ASN	114B	-42.422	43.300	-72.209	1.00	25.59	C
55	ATOM	2308	CG	ASN	114B	-41.958	43.794	-73.570	1.00	6.04	O
	ATOM	2309	OD1	ASN	114B	-41.706	42.998	-74.483	1.00	8.20	N
	ATOM	2310	ND2	ASN	114B	-41.847	45.107	-73.716	1.00	26.71	C
	ATOM	2311	C	ASN	114B	-44.874	43.519	-71.752	1.00	11.42	C
	ATOM	2312	O	ASN	114B	-45.242	43.449	-70.576	1.00	4.39	O
60	ATOM	2313	N	GLY	115B	-45.390	44.384	-72.623	1.00	6.57	C
	ATOM	2314	CA	GLY	115B	-46.445	45.295	-72.218	1.00	4.39	C
	ATOM	2315	C	GLY	115B	-47.737	44.546	-71.948	1.00	4.39	C

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	ATOM	2316	O	GLY	115B	-48.354	44.707	-70.895	1.00	4.47	O
	ATOM	2317	N	LEU	116B	-48.154	43.722	-72.901	1.00	5.66	N
	ATOM	2318	CA	LEU	116B	-49.377	42.946	-72.733	1.00	5.91	C
5	ATOM	2319	CB	LEU	116B	-49.658	42.108	-73.982	1.00	9.34	C
	ATOM	2320	CG	LEU	116B	-50.017	42.843	-75.276	1.00	12.19	C
	ATOM	2321	CD1	LEU	116B	-50.256	41.820	-76.381	1.00	13.60	C
	ATOM	2322	CD2	LEU	116B	-51.258	43.707	-75.064	1.00	12.16	C
	ATOM	2323	C	LEU	116B	-49.247	42.027	-71.517	1.00	5.65	C
10	ATOM	2324	O	LEU	116B	-50.198	41.838	-70.759	1.00	4.54	O
	ATOM	2325	N	LYS	117B	-48.061	41.462	-71.330	1.00	4.02	N
	ATOM	2326	CA	LYS	117B	-47.831	40.574	-70.201	1.00	4.05	C
	ATOM	2327	CB	LYS	117B	-46.386	40.071	-70.202	1.00	4.86	C
	ATOM	2328	CG	LYS	117B	-46.053	39.194	-69.006	1.00	8.47	C
15	ATOM	2329	CD	LYS	117B	-44.718	38.488	-69.188	1.00	7.05	C
	ATOM	2330	CE	LYS	117B	-43.566	39.463	-69.370	1.00	8.62	C
	ATOM	2331	NZ	LYS	117B	-42.313	38.741	-69.740	1.00	13.80	N
	ATOM	2332	C	LYS	117B	-48.131	41.261	-68.876	1.00	5.02	C
	ATOM	2333	O	LYS	117B	-48.867	40.721	-68.041	1.00	5.03	O
20	ATOM	2334	N	ASP	118B	-47.587	42.465	-68.694	1.00	3.29	N
	ATOM	2335	CA	ASP	118B	-47.798	43.196	-67.455	1.00	3.14	C
	ATOM	2336	CB	ASP	118B	-46.904	44.433	-67.387	1.00	2.01	C
	ATOM	2337	CG	ASP	118B	-46.802	44.986	-65.980	1.00	4.42	C
	ATOM	2338	OD1	ASP	118B	-46.351	44.237	-65.086	1.00	3.69	O
25	ATOM	2339	OD2	ASP	118B	-47.177	46.154	-65.756	1.00	3.76	O
	ATOM	2340	C	ASP	118B	-49.250	43.620	-67.283	1.00	2.90	C
	ATOM	2341	O	ASP	118B	-49.749	43.701	-66.161	1.00	3.26	O
	ATOM	2342	N	ILE	119B	-49.925	43.909	-68.390	1.00	2.60	N
	ATOM	2343	CA	ILE	119B	-51.318	44.307	-68.300	1.00	2.33	C
30	ATOM	2344	CB	ILE	119B	-51.832	44.853	-69.645	1.00	1.81	C
	ATOM	2345	CG2	ILE	119B	-53.340	45.001	-69.596	1.00	1.45	C
	ATOM	2346	CG1	ILE	119B	-51.148	46.195	-69.940	1.00	1.00	C
	ATOM	2347	CD1	ILE	119B	-51.487	46.805	-71.295	1.00	1.90	C
	ATOM	2348	C	ILE	119B	-52.153	43.114	-67.845	1.00	3.28	C
35	ATOM	2349	O	ILE	119B	-52.981	43.235	-66.945	1.00	1.53	O
	ATOM	2350	N	ILE	120B	-51.922	41.960	-68.457	1.00	4.47	N
	ATOM	2351	CA	ILE	120B	-52.650	40.754	-68.073	1.00	7.07	C
	ATOM	2352	CB	ILE	120B	-52.214	39.542	-68.923	1.00	6.84	C
	ATOM	2353	CG2	ILE	120B	-52.792	38.255	-68.338	1.00	9.94	C
40	ATOM	2354	CG1	ILE	120B	-52.672	39.730	-70.369	1.00	8.07	C
	ATOM	2355	CD1	ILE	120B	-52.164	38.659	-71.322	1.00	8.00	C
	ATOM	2356	C	ILE	120B	-52.383	40.456	-66.599	1.00	8.10	C
	ATOM	2357	O	ILE	120B	-53.295	40.109	-65.850	1.00	10.68	O
	ATOM	2358	N	ALA	121B	-51.131	40.611	-66.183	1.00	10.38	N
45	ATOM	2359	CA	ALA	121B	-50.753	40.353	-64.795	1.00	10.80	C
	ATOM	2360	CB	ALA	121B	-49.242	40.424	-64.651	1.00	8.94	C
	ATOM	2361	C	ALA	121B	-51.412	41.325	-63.820	1.00	11.72	C
	ATOM	2362	O	ALA	121B	-51.958	40.916	-62.796	1.00	12.69	O
	ATOM	2363	N	GLN	122B	-51.373	42.611	-64.149	1.00	13.51	N
50	ATOM	2364	CA	GLN	122B	-51.934	43.644	-63.288	1.00	17.15	C
	ATOM	2365	CB	GLN	122B	-51.478	45.020	-63.780	1.00	19.73	C
	ATOM	2366	CG	GLN	122B	-51.375	46.081	-62.696	1.00	24.59	C
	ATOM	2367	CD	GLN	122B	-50.388	45.711	-61.602	1.00	27.10	C
	ATOM	2368	OE1	GLN	122B	-50.634	44.804	-60.808	1.00	27.53	O
55	ATOM	2369	NE2	GLN	122B	-49.261	46.415	-61.558	1.00	27.90	N
	ATOM	2370	C	GLN	122B	-53.458	43.616	-63.168	1.00	18.50	C
	ATOM	2371	O	GLN	122B	-54.007	44.025	-62.145	1.00	19.68	O
	ATOM	2372	N	LEU	123B	-54.143	43.140	-64.203	1.00	17.95	N
	ATOM	2373	CA	LEU	123B	-55.601	43.087	-64.170	1.00	21.05	C
	ATOM	2374	CB	LEU	123B	-56.171	43.214	-65.586	1.00	16.96	C
60	ATOM	2375	CG	LEU	123B	-55.964	44.576	-66.256	1.00	17.66	C
	ATOM	2376	CD1	LEU	123B	-56.508	44.543	-67.679	1.00	12.24	C

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	ATOM	2377	CD2	LEU	123B	-56.657	45.656	-65.439	1.00	15.19	C
	ATOM	2378	C	LEU	123B	-56.112	41.808	-63.515	1.00	24.78	C
	ATOM	2379	O	LEU	123B	-57.198	41.324	-63.838	1.00	24.24	O
5	ATOM	2380	N	GLY	124B	-55.327	41.273	-62.585	1.00	27.91	N
	ATOM	2381	CA	GLY	124B	-55.713	40.057	-61.893	1.00	32.42	C
	ATOM	2382	C	GLY	124B	-55.957	38.883	-62.822	1.00	35.00	C
	ATOM	2383	O	GLY	124B	-57.071	38.361	-62.892	1.00	36.71	O
	ATOM	2384	N	ASN	125B	-54.915	38.467	-63.536	1.00	37.45	N
10	ATOM	2385	CA	ASN	125B	-55.003	37.342	-64.466	1.00	38.98	C
	ATOM	2386	CB	ASN	125B	-55.249	36.039	-63.690	1.00	43.03	C
	ATOM	2387	CG	ASN	125B	-55.027	34.791	-64.540	1.00	46.70	C
	ATOM	2388	OD1	ASN	125B	-55.800	34.494	-65.452	1.00	48.47	O
	ATOM	2389	ND2	ASN	125B	-53.959	34.056	-64.239	1.00	48.29	N
15	ATOM	2390	C	ASN	125B	-56.104	37.552	-65.509	1.00	37.04	C
	ATOM	2391	O	ASN	125B	-56.509	36.613	-66.195	1.00	37.49	O
	ATOM	2392	N	ASN	126B	-56.584	38.787	-65.628	1.00	35.87	N
	ATOM	2393	CA	ASN	126B	-57.628	39.098	-66.599	1.00	35.01	C
	ATOM	2394	CB	ASN	126B	-58.524	40.233	-66.093	1.00	34.84	C
	ATOM	2395	CG	ASN	126B	-59.512	39.773	-65.038	1.00	34.58	C
20	ATOM	2396	OD1	ASN	126B	-60.270	40.574	-64.491	1.00	35.41	O
	ATOM	2397	ND2	ASN	126B	-59.514	38.476	-64.751	1.00	35.15	N
	ATOM	2398	C	ASN	126B	-57.036	39.483	-67.947	1.00	34.96	C
	ATOM	2399	O	ASN	126B	-56.297	40.460	-68.059	1.00	33.64	O
	ATOM	2400	N	ASN	127B	-57.370	38.703	-68.968	1.00	33.78	N
25	ATOM	2401	CA	ASN	127B	-56.882	38.953	-70.315	1.00	33.02	C
	ATOM	2402	CB	ASN	127B	-55.964	37.810	-70.764	1.00	35.98	C
	ATOM	2403	CG	ASN	127B	-56.668	36.462	-70.786	1.00	37.66	C
	ATOM	2404	OD1	ASN	127B	-56.054	35.435	-71.079	1.00	39.57	O
	ATOM	2405	ND2	ASN	127B	-57.959	36.458	-70.478	1.00	39.63	N
30	ATOM	2406	C	ASN	127B	-58.062	39.079	-71.268	1.00	29.95	C
	ATOM	2407	O	ASN	127B	-57.891	39.100	-72.487	1.00	31.65	O
	ATOM	2408	N	SER	128B	-59.260	39.168	-70.698	1.00	27.17	N
	ATOM	2409	CA	SER	128B	-60.478	39.276	-71.488	1.00	22.22	C
	ATOM	2410	CB	SER	128B	-61.699	38.919	-70.635	1.00	24.63	C
35	ATOM	2411	OG	SER	128B	-61.941	39.904	-69.645	1.00	27.45	O
	ATOM	2412	C	SER	128B	-60.662	40.666	-72.086	1.00	17.26	C
	ATOM	2413	O	SER	128B	-61.791	41.102	-72.316	1.00	15.41	O
	ATOM	2414	N	PHE	129B	-59.557	41.366	-72.330	1.00	12.07	N
	ATOM	2415	CA	PHE	129B	-59.642	42.689	-72.934	1.00	8.40	C
40	ATOM	2416	CB	PHE	129B	-58.677	43.671	-72.259	1.00	5.02	C
	ATOM	2417	CG	PHE	129B	-57.243	43.231	-72.271	1.00	4.53	C
	ATOM	2418	CD1	PHE	129B	-56.397	43.587	-73.314	1.00	3.53	C
	ATOM	2419	CD2	PHE	129B	-56.731	42.480	-71.220	1.00	3.19	C
	ATOM	2420	CE1	PHE	129B	-55.054	43.206	-73.303	1.00	3.46	C
45	ATOM	2421	CE2	PHE	129B	-55.391	42.093	-71.200	1.00	4.90	C
	ATOM	2422	CZ	PHE	129B	-54.553	42.459	-72.241	1.00	3.02	C
	ATOM	2423	C	PHE	129B	-59.339	42.555	-74.418	1.00	6.14	C
	ATOM	2424	O	PHE	129B	-58.816	41.539	-74.862	1.00	6.28	O
	ATOM	2425	N	HIS	130B	-59.672	43.577	-75.192	1.00	4.10	N
50	ATOM	2426	CA	HIS	130B	-59.458	43.513	-76.622	1.00	3.45	C
	ATOM	2427	CB	HIS	130B	-60.638	44.179	-77.327	1.00	5.34	C
	ATOM	2428	CG	HIS	130B	-61.962	43.647	-76.876	1.00	8.98	C
	ATOM	2429	CD2	HIS	130B	-62.657	42.552	-77.263	1.00	8.24	C
	ATOM	2430	ND1	HIS	130B	-62.690	44.222	-75.855	1.00	10.64	N
55	ATOM	2431	CE1	HIS	130B	-63.776	43.503	-75.634	1.00	9.87	C
	ATOM	2432	NE2	HIS	130B	-63.780	42.484	-76.474	1.00	12.29	N
	ATOM	2433	C	HIS	130B	-58.127	44.108	-77.053	1.00	3.41	C
	ATOM	2434	O	HIS	130B	-57.550	44.938	-76.350	1.00	1.40	O
	ATOM	2435	N	ARG	131B	-57.627	43.651	-78.196	1.00	3.01	N
60	ATOM	2436	CA	ARG	131B	-56.350	44.131	-78.698	1.00	2.63	C
	ATOM	2437	CB	ARG	131B	-55.258	43.066	-78.508	1.00	5.78	C

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	ATOM	2438	CG	ARG	131B	-55.131	42.487	-77.109	1.00	7.17	C
	ATOM	2439	CD	ARG	131B	-56.097	41.321	-76.911	1.00	9.66	C
	ATOM	2440	NE	ARG	131B	-55.919	40.650	-75.626	1.00	10.93	N
	ATOM	2441	CZ	ARG	131B	-54.884	39.874	-75.314	1.00	13.28	C
5	ATOM	2442	NH1	ARG	131B	-53.914	39.660	-76.194	1.00	11.77	N
	ATOM	2443	NH2	ARG	131B	-54.826	39.295	-74.119	1.00	13.11	N
	ATOM	2444	C	ARG	131B	-56.356	44.524	-80.170	1.00	4.24	C
	ATOM	2445	O	ARG	131B	-56.925	43.815	-81.011	1.00	2.91	O
	ATOM	2446	N	LEU	132B	-55.730	45.664	-80.465	1.00	3.65	N
10	ATOM	2447	CA	LEU	132B	-55.561	46.127	-81.837	1.00	1.00	C
	ATOM	2448	CB	LEU	132B	-55.796	47.643	-81.971	1.00	1.80	C
	ATOM	2449	CG	LEU	132B	-55.415	48.216	-83.348	1.00	1.00	C
	ATOM	2450	CD1	LEU	132B	-56.264	47.555	-84.426	1.00	1.28	C
	ATOM	2451	CD2	LEU	132B	-55.606	49.750	-83.370	1.00	1.00	C
15	ATOM	2452	C	LEU	132B	-54.098	45.787	-82.132	1.00	2.48	C
	ATOM	2453	O	LEU	132B	-53.171	46.391	-81.581	1.00	1.00	O
	ATOM	2454	N	ARG	133B	-53.898	44.785	-82.978	1.00	1.00	N
	ATOM	2455	CA	ARG	133B	-52.564	44.335	-83.338	1.00	1.82	C
	ATOM	2456	CB	ARG	133B	-52.592	42.824	-83.576	1.00	2.80	C
20	ATOM	2457	CG	ARG	133B	-53.305	42.062	-82.457	1.00	2.92	C
	ATOM	2458	CD	ARG	133B	-53.561	40.608	-82.824	1.00	5.02	C
	ATOM	2459	NE	ARG	133B	-52.331	39.828	-82.904	1.00	6.40	N
	ATOM	2460	CZ	ARG	133B	-52.283	38.552	-83.262	1.00	10.22	C
	ATOM	2461	NH1	ARG	133B	-53.398	37.906	-83.578	1.00	10.19	N
25	ATOM	2462	NH2	ARG	133B	-51.116	37.917	-83.300	1.00	12.60	N
	ATOM	2463	C	ARG	133B	-52.073	45.061	-84.582	1.00	2.03	C
	ATOM	2464	O	ARG	133B	-52.627	44.899	-85.667	1.00	3.66	O
	ATOM	2465	N	LEU	134B	-51.033	45.869	-84.413	1.00	1.00	N
	ATOM	2466	CA	LEU	134B	-50.463	46.625	-85.522	1.00	2.19	C
30	ATOM	2467	CB	LEU	134B	-50.168	48.067	-85.094	1.00	2.72	C
	ATOM	2468	CG	LEU	134B	-51.342	48.904	-84.575	1.00	5.54	C
	ATOM	2469	CD1	LEU	134B	-52.394	49.088	-85.666	1.00	3.29	C
	ATOM	2470	CD2	LEU	134B	-51.942	48.218	-83.373	1.00	4.70	C
	ATOM	2471	C	LEU	134B	-49.183	45.942	-85.965	1.00	2.04	C
35	ATOM	2472	O	LEU	134B	-48.174	45.963	-85.253	1.00	2.64	O
	ATOM	2473	N	GLY	135B	-49.231	45.325	-87.139	1.00	2.59	N
	ATOM	2474	CA	GLY	135B	-48.065	44.632	-87.656	1.00	2.15	C
	ATOM	2475	C	GLY	135B	-46.815	45.484	-87.791	1.00	2.34	C
	ATOM	2476	O	GLY	135B	-46.875	46.633	-88.219	1.00	5.39	O
40	ATOM	2477	N	ILE	136B	-45.674	44.914	-87.413	1.00	2.33	N
	ATOM	2478	CA	ILE	136B	-44.391	45.602	-87.520	1.00	2.37	C
	ATOM	2479	CB	ILE	136B	-43.865	46.092	-86.151	1.00	2.52	C
	ATOM	2480	CG2	ILE	136B	-44.721	47.236	-85.639	1.00	1.16	C
	ATOM	2481	CG1	ILE	136B	-43.827	44.932	-85.156	1.00	1.83	C
45	ATOM	2482	CD1	ILE	136B	-42.898	45.174	-83.982	1.00	4.09	C
	ATOM	2483	C	ILE	136B	-43.361	44.636	-88.092	1.00	2.50	C
	ATOM	2484	O	ILE	136B	-42.197	44.984	-88.253	1.00	2.54	O
	ATOM	2485	N	GLY	137B	-43.799	43.418	-88.391	1.00	4.78	N
	ATOM	2486	CA	GLY	137B	-42.889	42.425	-88.935	1.00	2.44	C
50	ATOM	2487	C	GLY	137B	-42.043	41.746	-87.870	1.00	1.61	C
	ATOM	2488	O	GLY	137B	-42.038	42.158	-86.704	1.00	1.06	O
	ATOM	2489	N	HIS	138B	-41.323	40.706	-88.281	1.00	1.34	N
	ATOM	2490	CA	HIS	138B	-40.461	39.935	-87.384	1.00	1.00	C
	ATOM	2491	CB	HIS	138B	-41.046	38.534	-87.168	1.00	1.67	C
55	ATOM	2492	CG	HIS	138B	-40.357	37.745	-86.096	1.00	1.76	C
	ATOM	2493	CD2	HIS	138B	-39.104	37.238	-86.027	1.00	3.34	C
	ATOM	2494	ND1	HIS	138B	-40.962	37.436	-84.896	1.00	4.71	N
	ATOM	2495	CE1	HIS	138B	-40.108	36.779	-84.131	1.00	3.61	C
	ATOM	2496	NE2	HIS	138B	-38.973	36.646	-84.794	1.00	3.60	N
60	ATOM	2497	C	HIS	138B	-39.071	39.813	-88.014	1.00	3.36	C
	ATOM	2498	O	HIS	138B	-38.950	39.554	-89.211	1.00	6.22	O

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	ATOM	2499	N	PRO	139B	-38.007	39.993	-87.213	1.00	4.22	N
	ATOM	2500	CD	PRO	139B	-38.061	40.396	-85.796	1.00	4.12	C
	ATOM	2501	CA	PRO	139B	-36.613	39.911	-87.669	1.00	4.56	C
	ATOM	2502	CB	PRO	139B	-35.837	40.481	-86.484	1.00	4.77	C
5	ATOM	2503	CG	PRO	139B	-36.671	40.053	-85.318	1.00	3.14	C
	ATOM	2504	C	PRO	139B	-36.114	38.521	-88.083	1.00	6.85	C
	ATOM	2505	O	PRO	139B	-35.112	38.404	-88.785	1.00	5.91	O
	ATOM	2506	N	GLY	140B	-36.802	37.472	-87.653	1.00	8.55	N
	ATOM	2507	CA	GLY	140B	-36.384	36.130	-88.016	1.00	11.48	C
10	ATOM	2508	C	GLY	140B	-36.188	35.205	-86.831	1.00	13.15	C
	ATOM	2509	O	GLY	140B	-36.931	34.235	-86.661	1.00	16.89	O
	ATOM	2510	N	HIS	141B	-35.193	35.497	-86.003	1.00	14.08	N
	ATOM	2511	CA	HIS	141B	-34.922	34.669	-84.837	1.00	12.93	C
	ATOM	2512	CB	HIS	141B	-33.430	34.339	-84.758	1.00	13.74	C
15	ATOM	2513	CG	HIS	141B	-33.112	33.252	-83.781	1.00	13.95	C
	ATOM	2514	CD2	HIS	141B	-32.589	33.300	-82.535	1.00	15.78	C
	ATOM	2515	ND1	HIS	141B	-33.394	31.926	-84.028	1.00	13.89	N
	ATOM	2516	CE1	HIS	141B	-33.060	31.204	-82.974	1.00	14.40	C
	ATOM	2517	NE2	HIS	141B	-32.569	32.014	-82.053	1.00	16.37	N
20	ATOM	2518	C	HIS	141B	-35.360	35.381	-83.558	1.00	13.42	C
	ATOM	2519	O	HIS	141B	-35.219	36.596	-83.436	1.00	14.28	O
	ATOM	2520	N	SER	142B	-35.891	34.617	-82.608	1.00	14.38	N
	ATOM	2521	CA	SER	142B	-36.360	35.177	-81.342	1.00	13.26	C
	ATOM	2522	CB	SER	142B	-36.788	34.057	-80.393	1.00	12.76	C
25	ATOM	2523	OG	SER	142B	-35.659	33.336	-79.921	1.00	13.41	O
	ATOM	2524	C	SER	142B	-35.289	36.019	-80.656	1.00	13.76	C
	ATOM	2525	O	SER	142B	-35.603	36.959	-79.921	1.00	12.08	O
	ATOM	2526	N	SER	143B	-34.027	35.674	-80.895	1.00	13.86	N
	ATOM	2527	CA	SER	143B	-32.908	36.389	-80.290	1.00	13.82	C
30	ATOM	2528	CB	SER	143B	-31.601	35.637	-80.566	1.00	15.38	C
	ATOM	2529	OG	SER	143B	-30.526	36.196	-79.834	1.00	20.82	O
	ATOM	2530	C	SER	143B	-32.772	37.832	-80.780	1.00	12.00	C
	ATOM	2531	O	SER	143B	-32.217	38.680	-80.074	1.00	9.61	O
	ATOM	2532	N	LEU	144B	-33.277	38.107	-81.981	1.00	10.29	N
35	ATOM	2533	CA	LEU	144B	-33.187	39.441	-82.575	1.00	8.43	C
	ATOM	2534	CB	LEU	144B	-33.102	39.320	-84.102	1.00	8.85	C
	ATOM	2535	CG	LEU	144B	-31.904	38.586	-84.722	1.00	10.18	C
	ATOM	2536	CD1	LEU	144B	-32.102	38.480	-86.229	1.00	11.73	C
	ATOM	2537	CD2	LEU	144B	-30.616	39.336	-84.412	1.00	11.75	C
40	ATOM	2538	C	LEU	144B	-34.339	40.389	-82.220	1.00	6.83	C
	ATOM	2539	O	LEU	144B	-34.245	41.597	-82.441	1.00	7.49	O
	ATOM	2540	N	VAL	145B	-35.411	39.838	-81.665	1.00	5.18	N
	ATOM	2541	CA	VAL	145B	-36.599	40.604	-81.311	1.00	4.97	C
	ATOM	2542	CB	VAL	145B	-37.629	39.694	-80.593	1.00	3.62	C
45	ATOM	2543	CG1	VAL	145B	-38.841	40.515	-80.145	1.00	3.67	C
	ATOM	2544	CG2	VAL	145B	-38.066	38.583	-81.532	1.00	6.84	C
	ATOM	2545	C	VAL	145B	-36.369	41.865	-80.474	1.00	4.98	C
	ATOM	2546	O	VAL	145B	-36.891	42.932	-80.805	1.00	3.64	O
	ATOM	2547	N	SER	146B	-35.597	41.760	-79.397	1.00	5.06	N
50	ATOM	2548	CA	SER	146B	-35.347	42.932	-78.560	1.00	6.97	C
	ATOM	2549	CB	SER	146B	-34.431	42.574	-77.388	1.00	9.91	C
	ATOM	2550	OG	SER	146B	-35.131	41.798	-76.432	1.00	12.54	O
	ATOM	2551	C	SER	146B	-34.758	44.111	-79.327	1.00	8.11	C
	ATOM	2552	O	SER	146B	-35.273	45.228	-79.254	1.00	8.37	O
55	ATOM	2553	N	GLY	147B	-33.678	43.873	-80.062	1.00	5.19	N
	ATOM	2554	CA	GLY	147B	-33.073	44.954	-80.817	1.00	6.87	C
	ATOM	2555	C	GLY	147B	-33.967	45.453	-81.937	1.00	4.82	C
	ATOM	2556	O	GLY	147B	-33.955	46.629	-82.281	1.00	5.54	O
	ATOM	2557	N	TYR	148B	-34.753	44.549	-82.504	1.00	3.86	N
60	ATOM	2558	CA	TYR	148B	-35.649	44.887	-83.602	1.00	2.80	C
	ATOM	2559	CB	TYR	148B	-36.211	43.598	-84.208	1.00	2.05	C

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	ATOM	2560	CG	TYR	148B	-37.141	43.798	-85.382	1.00	3.38	C
	ATOM	2561	CD1	TYR	148B	-36.642	43.973	-86.674	1.00	3.49	C
	ATOM	2562	CE1	TYR	148B	-37.501	44.121	-87.760	1.00	2.93	C
	ATOM	2563	CD2	TYR	148B	-38.525	43.781	-85.204	1.00	1.87	C
5	ATOM	2564	CE2	TYR	148B	-39.394	43.932	-86.282	1.00	1.52	C
	ATOM	2565	CZ	TYR	148B	-38.877	44.099	-87.555	1.00	3.36	C
	ATOM	2566	OH	TYR	148B	-39.741	44.229	-88.621	1.00	3.17	O
	ATOM	2567	C	TYR	148B	-36.805	45.813	-83.215	1.00	2.27	C
	ATOM	2568	O	TYR	148B	-37.066	46.793	-83.913	1.00	1.00	O
	ATOM	2569	N	VAL	149B	-37.504	45.512	-82.120	1.00	1.46	N
10	ATOM	2570	CA	VAL	149B	-38.626	46.358	-81.724	1.00	1.59	C
	ATOM	2571	CB	VAL	149B	-39.600	45.645	-80.752	1.00	3.52	C
	ATOM	2572	CG1	VAL	149B	-40.096	44.345	-81.380	1.00	1.00	C
	ATOM	2573	CG2	VAL	149B	-38.923	45.388	-79.412	1.00	3.43	C
15	ATOM	2574	C	VAL	149B	-38.166	47.660	-81.087	1.00	2.32	C
	ATOM	2575	O	VAL	149B	-38.938	48.602	-80.979	1.00	1.77	O
	ATOM	2576	N	LEU	150B	-36.905	47.718	-80.676	1.00	1.60	N
	ATOM	2577	CA	LEU	150B	-36.389	48.942	-80.068	1.00	3.12	C
20	ATOM	2578	CB	LEU	150B	-35.413	48.606	-78.933	1.00	3.24	C
	ATOM	2579	CG	LEU	150B	-36.091	47.862	-77.770	1.00	3.19	C
	ATOM	2580	CD1	LEU	150B	-35.112	47.691	-76.626	1.00	5.08	C
	ATOM	2581	CD2	LEU	150B	-37.321	48.623	-77.306	1.00	5.58	C
25	ATOM	2582	C	LEU	150B	-35.709	49.796	-81.127	1.00	2.77	C
	ATOM	2583	O	LEU	150B	-35.108	50.824	-80.825	1.00	3.41	O
	ATOM	2584	N	GLY	151B	-35.824	49.367	-82.379	1.00	2.18	N
	ATOM	2585	CA	GLY	151B	-35.224	50.111	-83.467	1.00	3.00	C
30	ATOM	2586	C	GLY	151B	-36.236	50.922	-84.255	1.00	2.51	C
	ATOM	2587	O	GLY	151B	-37.448	50.706	-84.161	1.00	1.00	O
	ATOM	2588	N	ALA	152B	-35.731	51.878	-85.026	1.00	2.34	N
	ATOM	2589	CA	ALA	152B	-36.576	52.729	-85.855	1.00	4.72	C
35	ATOM	2590	CB	ALA	152B	-35.951	54.120	-85.982	1.00	4.78	C
	ATOM	2591	C	ALA	152B	-36.688	52.076	-87.225	1.00	4.69	C
	ATOM	2592	O	ALA	152B	-35.683	51.877	-87.904	1.00	7.78	O
	ATOM	2593	N	ALA	153B	-37.907	51.742	-87.631	1.00	5.53	N
40	ATOM	2594	CA	ALA	153B	-38.118	51.097	-88.921	1.00	5.80	C
	ATOM	2595	CB	ALA	153B	-39.550	50.590	-89.018	1.00	4.77	C
	ATOM	2596	C	ALA	153B	-37.816	52.021	-90.097	1.00	6.57	C
	ATOM	2597	O	ALA	153B	-37.958	53.239	-90.000	1.00	5.67	O
45	ATOM	2598	N	PRO	154B	-37.366	51.450	-91.223	1.00	9.25	N
	ATOM	2599	CD	PRO	154B	-37.023	50.042	-91.483	1.00	10.03	C
	ATOM	2600	CA	PRO	154B	-37.070	52.284	-92.390	1.00	9.15	C
	ATOM	2601	CB	PRO	154B	-36.507	51.281	-93.401	1.00	10.04	C
50	ATOM	2602	CG	PRO	154B	-37.107	49.979	-92.983	1.00	10.60	C
	ATOM	2603	C	PRO	154B	-38.350	52.964	-92.878	1.00	9.66	C
	ATOM	2604	O	PRO	154B	-39.458	52.539	-92.540	1.00	8.06	O
	ATOM	2605	N	ARG	155B	-38.186	54.015	-93.670	1.00	10.12	N
55	ATOM	2606	CA	ARG	155B	-39.307	54.776	-94.200	1.00	14.62	C
	ATOM	2607	CB	ARG	155B	-38.799	55.732	-95.279	1.00	18.36	C
	ATOM	2608	CG	ARG	155B	-39.867	56.602	-95.900	1.00	24.01	C
	ATOM	2609	CD	ARG	155B	-40.096	56.234	-97.357	1.00	28.91	C
60	ATOM	2610	NE	ARG	155B	-40.781	54.952	-97.524	1.00	32.92	N
	ATOM	2611	CZ	ARG	155B	-41.153	54.460	-98.702	1.00	33.54	C
	ATOM	2612	NH1	ARG	155B	-40.903	55.142	-99.807	1.00	34.84	N
	ATOM	2613	NH2	ARG	155B	-41.784	53.295	-98.781	1.00	34.76	N
65	ATOM	2614	C	ARG	155B	-40.447	53.922	-94.757	1.00	11.53	C
	ATOM	2615	O	ARG	155B	-41.610	54.127	-94.408	1.00	12.22	O
	ATOM	2616	N	SER	156B	-40.114	52.964	-95.616	1.00	10.15	N
	ATOM	2617	CA	SER	156B	-41.126	52.106	-96.224	1.00	8.14	C
70	ATOM	2618	CB	SER	156B	-40.465	51.057	-97.124	1.00	7.58	C
	ATOM	2619	OG	SER	156B	-39.666	50.154	-96.379	1.00	9.39	O
	ATOM	2620	C	SER	156B	-42.011	51.406	-95.200	1.00	7.78	C

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	ATOM	2621	O	SER	156B	-43.225	51.330	-95.365	1.00	7.67	O
	ATOM	2622	N	GLU	157B	-41.399	50.889	-94.143	1.00	7.58	N
	ATOM	2623	CA	GLU	157B	-42.153	50.190	-93.117	1.00	5.79	C
	ATOM	2624	CB	GLU	157B	-41.197	49.401	-92.229	1.00	4.06	C
5	ATOM	2625	CG	GLU	157B	-40.617	48.190	-92.931	1.00	5.98	C
	ATOM	2626	CD	GLU	157B	-39.546	47.496	-92.113	1.00	6.79	C
	ATOM	2627	OE1	GLU	157B	-39.700	47.412	-90.874	1.00	7.67	O
	ATOM	2628	OE2	GLU	157B	-38.559	47.024	-92.710	1.00	8.34	O
	ATOM	2629	C	GLU	157B	-43.002	51.138	-92.283	1.00	5.80	C
10	ATOM	2630	O	GLU	157B	-44.121	50.808	-91.901	1.00	5.45	O
	ATOM	2631	N	GLN	158B	-42.477	52.323	-92.001	1.00	5.94	N
	ATOM	2632	CA	GLN	158B	-43.245	53.275	-91.217	1.00	7.29	C
	ATOM	2633	CB	GLN	158B	-42.376	54.461	-90.818	1.00	8.03	C
	ATOM	2634	CG	GLN	158B	-41.366	54.075	-89.751	1.00	13.11	C
15	ATOM	2635	CD	GLN	158B	-40.689	55.268	-89.115	1.00	14.69	C
	ATOM	2636	OE1	GLN	158B	-41.304	56.324	-88.930	1.00	18.82	O
	ATOM	2637	NE2	GLN	158B	-39.420	55.101	-88.755	1.00	13.99	N
	ATOM	2638	C	GLN	158B	-44.477	53.733	-91.980	1.00	5.65	C
	ATOM	2639	O	GLN	158B	-45.522	53.964	-91.381	1.00	4.85	O
20	ATOM	2640	N	GLU	159B	-44.360	53.848	-93.301	1.00	7.12	N
	ATOM	2641	CA	GLU	159B	-45.499	54.254	-94.123	1.00	8.74	C
	ATOM	2642	CB	GLU	159B	-45.111	54.382	-95.596	1.00	11.33	C
	ATOM	2643	CG	GLU	159B	-43.942	55.299	-95.892	1.00	15.61	C
	ATOM	2644	CD	GLU	159B	-43.862	55.661	-97.371	1.00	19.50	C
25	ATOM	2645	OE1	GLU	159B	-42.956	56.438	-97.745	1.00	21.70	O
	ATOM	2646	OE2	GLU	159B	-44.712	55.173	-98.156	1.00	18.76	O
	ATOM	2647	C	GLU	159B	-46.595	53.202	-94.014	1.00	8.03	C
	ATOM	2648	O	GLU	159B	-47.769	53.530	-93.835	1.00	8.03	O
	ATOM	2649	N	LEU	160B	-46.209	51.934	-94.141	1.00	7.01	N
30	ATOM	2650	CA	LEU	160B	-47.162	50.833	-94.055	1.00	6.37	C
	ATOM	2651	CB	LEU	160B	-46.454	49.500	-94.338	1.00	6.74	C
	ATOM	2652	CG	LEU	160B	-45.915	49.403	-95.770	1.00	8.35	C
	ATOM	2653	CD1	LEU	160B	-45.150	48.105	-95.962	1.00	5.59	C
	ATOM	2654	CD2	LEU	160B	-47.079	49.492	-96.751	1.00	5.33	C
35	ATOM	2655	C	LEU	160B	-47.844	50.794	-92.688	1.00	5.31	C
	ATOM	2656	O	LEU	160B	-49.048	50.541	-92.589	1.00	3.17	O
	ATOM	2657	N	LEU	161B	-47.083	51.058	-91.633	1.00	2.65	N
	ATOM	2658	CA	LEU	161B	-47.661	51.052	-90.297	1.00	3.94	C
	ATOM	2659	CB	LEU	161B	-46.594	51.319	-89.233	1.00	4.42	C
40	ATOM	2660	CG	LEU	161B	-46.784	50.716	-87.836	1.00	7.69	C
	ATOM	2661	CD1	LEU	161B	-45.839	51.430	-86.884	1.00	8.56	C
	ATOM	2662	CD2	LEU	161B	-48.218	50.841	-87.345	1.00	9.84	C
	ATOM	2663	C	LEU	161B	-48.715	52.154	-90.243	1.00	2.84	C
	ATOM	2664	O	LEU	161B	-49.825	51.929	-89.771	1.00	5.39	O
45	ATOM	2665	N	ASP	162B	-48.361	53.343	-90.730	1.00	5.24	N
	ATOM	2666	CA	ASP	162B	-49.297	54.470	-90.736	1.00	5.75	C
	ATOM	2667	CB	ASP	162B	-48.711	55.696	-91.456	1.00	6.09	C
	ATOM	2668	CG	ASP	162B	-47.504	56.293	-90.749	1.00	9.13	C
	ATOM	2669	OD1	ASP	162B	-47.408	56.227	-89.505	1.00	8.65	O
50	ATOM	2670	OD2	ASP	162B	-46.648	56.860	-91.458	1.00	8.00	O
	ATOM	2671	C	ASP	162B	-50.603	54.096	-91.440	1.00	8.19	C
	ATOM	2672	O	ASP	162B	-51.696	54.408	-90.955	1.00	6.77	O
	ATOM	2673	N	GLU	163B	-50.475	53.448	-92.595	1.00	7.86	N
	ATOM	2674	CA	GLU	163B	-51.625	53.027	-93.385	1.00	8.90	C
55	ATOM	2675	CB	GLU	163B	-51.148	52.350	-94.682	1.00	13.74	C
	ATOM	2676	CG	GLU	163B	-50.443	53.306	-95.646	1.00	17.83	C
	ATOM	2677	CD	GLU	163B	-49.594	52.603	-96.705	1.00	24.48	C
	ATOM	2678	OE1	GLU	163B	-50.120	51.754	-97.456	1.00	26.96	O
	ATOM	2679	OE2	GLU	163B	-48.387	52.912	-96.793	1.00	27.42	O
60	ATOM	2680	C	GLU	163B	-52.495	52.079	-92.569	1.00	8.54	C
	ATOM	2681	O	GLU	163B	-53.718	52.222	-92.534	1.00	6.69	O

	ATOM	2682	N	SER	164B	-51.850	51.128	-91.900	1.00	6.40	N
	ATOM	2683	CA	SER	164B	-52.539	50.153	-91.062	1.00	4.06	C
	ATOM	2684	CB	SER	164B	-51.521	49.183	-90.456	1.00	5.96	C
	ATOM	2685	OG	SER	164B	-52.153	48.020	-89.957	1.00	10.66	O
5	ATOM	2686	C	SER	164B	-53.297	50.880	-89.946	1.00	4.01	C
	ATOM	2687	O	SER	164B	-54.412	50.496	-89.581	1.00	4.23	O
	ATOM	2688	N	ILE	165B	-52.682	51.930	-89.407	1.00	2.40	N
	ATOM	2689	CA	ILE	165B	-53.300	52.729	-88.350	1.00	2.62	C
	ATOM	2690	CB	ILE	165B	-52.288	53.737	-87.757	1.00	3.48	C
10	ATOM	2691	CG2	ILE	165B	-53.020	54.887	-87.073	1.00	4.52	C
	ATOM	2692	CG1	ILE	165B	-51.353	53.001	-86.787	1.00	4.29	C
	ATOM	2693	CD1	ILE	165B	-50.159	53.827	-86.308	1.00	3.59	C
	ATOM	2694	C	ILE	165B	-54.507	53.474	-88.915	1.00	4.67	C
	ATOM	2695	O	ILE	165B	-55.540	53.615	-88.253	1.00	4.73	O
15	ATOM	2696	N	ASP	166B	-54.375	53.953	-90.144	1.00	4.58	N
	ATOM	2697	CA	ASP	166B	-55.482	54.650	-90.767	1.00	5.60	C
	ATOM	2698	CB	ASP	166B	-55.065	55.231	-92.118	1.00	8.09	C
	ATOM	2699	CG	ASP	166B	-56.092	56.196	-92.661	1.00	10.65	C
	ATOM	2700	OD1	ASP	166B	-56.461	57.126	-91.918	1.00	10.81	O
20	ATOM	2701	OD2	ASP	166B	-56.530	56.028	-93.817	1.00	16.06	O
	ATOM	2702	C	ASP	166B	-56.631	53.661	-90.947	1.00	6.94	C
	ATOM	2703	O	ASP	166B	-57.795	54.005	-90.730	1.00	3.90	O
	ATOM	2704	N	PHE	167B	-56.292	52.434	-91.346	1.00	5.50	N
	ATOM	2705	CA	PHE	167B	-57.275	51.369	-91.543	1.00	7.94	C
25	ATOM	2706	CB	PHE	167B	-56.583	50.053	-91.920	1.00	11.88	C
	ATOM	2707	CG	PHE	167B	-56.127	49.969	-93.352	1.00	15.37	C
	ATOM	2708	CD1	PHE	167B	-55.297	48.930	-93.756	1.00	16.25	C
	ATOM	2709	CD2	PHE	167B	-56.546	50.894	-94.299	1.00	16.58	C
	ATOM	2710	CE1	PHE	167B	-54.892	48.811	-95.083	1.00	18.05	C
30	ATOM	2711	CE2	PHE	167B	-56.146	50.783	-95.630	1.00	18.43	C
	ATOM	2712	CZ	PHE	167B	-55.320	49.742	-96.022	1.00	17.69	C
	ATOM	2713	C	PHE	167B	-58.052	51.131	-90.256	1.00	5.81	C
	ATOM	2714	O	PHE	167B	-59.275	51.003	-90.267	1.00	7.01	O
	ATOM	2715	N	ALA	168B	-57.324	51.037	-89.148	1.00	4.56	N
35	ATOM	2716	CA	ALA	168B	-57.940	50.806	-87.847	1.00	4.69	C
	ATOM	2717	CB	ALA	168B	-56.862	50.596	-86.788	1.00	3.98	C
	ATOM	2718	C	ALA	168B	-58.859	51.962	-87.451	1.00	3.13	C
	ATOM	2719	O	ALA	168B	-59.947	51.744	-86.921	1.00	2.35	O
	ATOM	2720	N	LEU	169B	-58.430	53.194	-87.708	1.00	3.70	N
40	ATOM	2721	CA	LEU	169B	-59.253	54.349	-87.367	1.00	4.06	C
	ATOM	2722	CB	LEU	169B	-58.493	55.646	-87.631	1.00	3.86	C
	ATOM	2723	CG	LEU	169B	-57.397	55.939	-86.601	1.00	6.77	C
	ATOM	2724	CD1	LEU	169B	-56.620	57.168	-87.019	1.00	7.96	C
	ATOM	2725	CD2	LEU	169B	-58.018	56.138	-85.228	1.00	6.93	C
45	ATOM	2726	C	LEU	169B	-60.538	54.319	-88.179	1.00	5.26	C
	ATOM	2727	O	LEU	169B	-61.565	54.857	-87.762	1.00	5.46	O
	ATOM	2728	N	GLY	170B	-60.469	53.670	-89.337	1.00	6.56	N
	ATOM	2729	CA	GLY	170B	-61.629	53.553	-90.200	1.00	6.37	C
	ATOM	2730	C	GLY	170B	-62.757	52.756	-89.566	1.00	8.05	C
50	ATOM	2731	O	GLY	170B	-63.925	52.972	-89.890	1.00	9.59	O
	ATOM	2732	N	VAL	171B	-62.426	51.827	-88.672	1.00	6.07	N
	ATOM	2733	CA	VAL	171B	-63.460	51.036	-88.017	1.00	3.68	C
	ATOM	2734	CB	VAL	171B	-63.154	49.516	-88.086	1.00	4.84	C
	ATOM	2735	CG1	VAL	171B	-63.032	49.088	-89.533	1.00	5.50	C
55	ATOM	2736	CG2	VAL	171B	-61.879	49.187	-87.317	1.00	6.04	C
	ATOM	2737	C	VAL	171B	-63.644	51.461	-86.559	1.00	3.29	C
	ATOM	2738	O	VAL	171B	-64.084	50.679	-85.725	1.00	1.94	O
	ATOM	2739	N	LEU	172B	-63.298	52.710	-86.258	1.00	2.10	N
	ATOM	2740	CA	LEU	172B	-63.456	53.238	-84.909	1.00	1.93	C
60	ATOM	2741	CB	LEU	172B	-62.961	54.687	-84.838	1.00	1.44	C
	ATOM	2742	CG	LEU	172B	-63.069	55.405	-83.490	1.00	2.60	C

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	ATOM	2743	CD1	LEU	172B	-62.275	54.660	-82.440	1.00	1.64	C
	ATOM	2744	CD2	LEU	172B	-62.532	56.831	-83.620	1.00	1.75	C
	ATOM	2745	C	LEU	172B	-64.920	53.185	-84.472	1.00	2.63	C
	ATOM	2746	O	LEU	172B	-65.212	52.896	-83.314	1.00	3.20	O
5	ATOM	2747	N	PRO	173B	-65.864	53.471	-85.390	1.00	2.56	N
	ATOM	2748	CD	PRO	173B	-65.737	53.897	-86.795	1.00	1.80	C
	ATOM	2749	CA	PRO	173B	-67.280	53.426	-84.991	1.00	2.37	C
	ATOM	2750	CB	PRO	173B	-68.020	53.683	-86.304	1.00	3.04	C
	ATOM	2751	CG	PRO	173B	-67.082	54.565	-87.050	1.00	2.88	C
10	ATOM	2752	C	PRO	173B	-67.660	52.077	-84.377	1.00	2.86	C
	ATOM	2753	O	PRO	173B	-68.306	52.019	-83.327	1.00	1.83	O
	ATOM	2754	N	GLU	174B	-67.259	50.990	-85.031	1.00	3.72	N
	ATOM	2755	CA	GLU	174B	-67.577	49.660	-84.524	1.00	3.38	C
	ATOM	2756	CB	GLU	174B	-67.281	48.583	-85.575	1.00	5.65	C
15	ATOM	2757	CG	GLU	174B	-68.244	48.577	-86.753	1.00	6.38	C
	ATOM	2758	CD	GLU	174B	-67.973	49.684	-87.757	1.00	7.10	C
	ATOM	2759	OE1	GLU	174B	-68.807	49.866	-88.671	1.00	5.12	O
	ATOM	2760	OE2	GLU	174B	-66.933	50.368	-87.642	1.00	3.69	O
	ATOM	2761	C	GLU	174B	-66.814	49.340	-83.244	1.00	4.35	C
20	ATOM	2762	O	GLU	174B	-67.377	48.778	-82.302	1.00	5.56	O
	ATOM	2763	N	MET	175B	-65.533	49.691	-83.200	1.00	5.24	N
	ATOM	2764	CA	MET	175B	-64.764	49.411	-82.000	1.00	3.56	C
	ATOM	2765	CB	MET	175B	-63.300	49.807	-82.195	1.00	3.91	C
	ATOM	2766	CG	MET	175B	-62.605	49.003	-83.284	1.00	3.27	C
25	ATOM	2767	SD	MET	175B	-60.815	49.165	-83.218	1.00	4.52	S
	ATOM	2768	CE	MET	175B	-60.632	50.873	-83.707	1.00	3.12	C
	ATOM	2769	C	MET	175B	-65.373	50.151	-80.809	1.00	3.88	C
	ATOM	2770	O	MET	175B	-65.578	49.566	-79.748	1.00	2.06	O
	ATOM	2771	N	LEU	176B	-65.688	51.432	-80.986	1.00	3.16	N
30	ATOM	2772	CA	LEU	176B	-66.267	52.187	-79.888	1.00	2.84	C
	ATOM	2773	CB	LEU	176B	-66.524	53.647	-80.299	1.00	4.21	C
	ATOM	2774	CG	LEU	176B	-65.291	54.540	-80.494	1.00	3.57	C
	ATOM	2775	CD1	LEU	176B	-65.743	55.927	-80.919	1.00	3.25	C
	ATOM	2776	CD2	LEU	176B	-64.488	54.629	-79.202	1.00	1.80	C
35	ATOM	2777	C	LEU	176B	-67.561	51.542	-79.413	1.00	2.48	C
	ATOM	2778	O	LEU	176B	-67.862	51.537	-78.223	1.00	3.56	O
	ATOM	2779	N	ALA	177B	-68.315	50.976	-80.347	1.00	2.70	N
	ATOM	2780	CA	ALA	177B	-69.581	50.343	-80.008	1.00	4.47	C
	ATOM	2781	CB	ALA	177B	-70.474	50.281	-81.234	1.00	3.49	C
40	ATOM	2782	C	ALA	177B	-69.393	48.949	-79.438	1.00	4.98	C
	ATOM	2783	O	ALA	177B	-70.305	48.398	-78.818	1.00	7.35	O
	ATOM	2784	N	GLY	178B	-68.212	48.380	-79.649	1.00	6.22	N
	ATOM	2785	CA	GLY	178B	-67.955	47.039	-79.166	1.00	6.15	C
	ATOM	2786	C	GLY	178B	-68.391	46.001	-80.184	1.00	6.08	C
45	ATOM	2787	O	GLY	178B	-68.512	44.817	-79.859	1.00	4.07	O
	ATOM	2788	N	ASP	179B	-68.633	46.441	-81.416	1.00	4.63	N
	ATOM	2789	CA	ASP	179B	-69.046	45.532	-82.481	1.00	6.11	C
	ATOM	2790	CB	ASP	179B	-69.922	46.277	-83.500	1.00	6.31	C
	ATOM	2791	CG	ASP	179B	-70.675	45.337	-84.427	1.00	7.98	C
50	ATOM	2792	OD1	ASP	179B	-71.540	45.820	-85.188	1.00	7.79	O
	ATOM	2793	OD2	ASP	179B	-70.403	44.119	-84.402	1.00	8.57	O
	ATOM	2794	C	ASP	179B	-67.796	44.960	-83.146	1.00	6.31	C
	ATOM	2795	O	ASP	179B	-67.450	45.303	-84.280	1.00	3.63	O
	ATOM	2796	N	TRP	180B	-67.124	44.070	-82.425	1.00	7.68	N
55	ATOM	2797	CA	TRP	180B	-65.896	43.459	-82.909	1.00	8.01	C
	ATOM	2798	CB	TRP	180B	-65.261	42.645	-81.781	1.00	7.72	C
	ATOM	2799	CG	TRP	180B	-65.109	43.469	-80.535	1.00	6.32	C
	ATOM	2800	CD2	TRP	180B	-64.208	44.564	-80.338	1.00	5.07	C
	ATOM	2801	CE2	TRP	180B	-64.452	45.077	-79.047	1.00	3.64	C
60	ATOM	2802	CB3	TRP	180B	-63.217	45.162	-81.130	1.00	5.34	C
	ATOM	2803	CD1	TRP	180B	-65.842	43.366	-79.390	1.00	5.46	C

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	ATOM	2804	NE1	TRP	180B	-65.456	44.328	-78.492	1.00	4.66	N
	ATOM	2805	CZ2	TRP	180B	-63.742	46.162	-78.524	1.00	4.11	C
	ATOM	2806	CZ3	TRP	180B	-62.509	46.245	-80.612	1.00	6.60	C
	ATOM	2807	CH2	TRP	180B	-62.776	46.732	-79.319	1.00	5.80	C
5	ATOM	2808	C	TRP	180B	-66.071	42.605	-84.162	1.00	8.85	C
	ATOM	2809	O	TRP	180B	-65.218	42.624	-85.051	1.00	7.56	O
	ATOM	2810	N	THR	181B	-67.166	41.857	-84.240	1.00	10.79	N
	ATOM	2811	CA	THR	181B	-67.412	41.029	-85.418	1.00	12.35	C
	ATOM	2812	CB	THR	181B	-68.793	40.355	-85.353	1.00	13.87	C
	ATOM	2813	OG1	THR	181B	-69.810	41.359	-85.272	1.00	18.61	O
10	ATOM	2814	CG2	THR	181B	-68.888	39.459	-84.136	1.00	13.72	C
	ATOM	2815	C	THR	181B	-67.355	41.912	-86.663	1.00	10.61	C
	ATOM	2816	O	THR	181B	-66.607	41.633	-87.600	1.00	9.89	O
	ATOM	2817	N	ARG	182B	-68.139	42.989	-86.654	1.00	11.80	N
	ATOM	2818	CA	ARG	182B	-68.188	43.934	-87.768	1.00	11.96	C
15	ATOM	2819	CB	ARG	182B	-69.237	45.010	-87.490	1.00	18.03	C
	ATOM	2820	CG	ARG	182B	-70.312	45.158	-88.552	1.00	25.42	C
	ATOM	2821	CD	ARG	182B	-69.759	45.626	-89.887	1.00	31.52	C
	ATOM	2822	NE	ARG	182B	-70.822	46.173	-90.729	1.00	38.72	N
	ATOM	2823	CZ	ARG	182B	-70.653	46.617	-91.971	1.00	41.14	C
20	ATOM	2824	NH1	ARG	182B	-71.686	47.099	-92.648	1.00	43.26	N
	ATOM	2825	NH2	ARG	182B	-69.458	46.573	-92.543	1.00	42.09	N
	ATOM	2826	C	ARG	182B	-66.830	44.602	-87.972	1.00	11.16	C
	ATOM	2827	O	ARG	182B	-66.341	44.723	-89.097	1.00	9.95	O
	ATOM	2828	N	ALA	183B	-66.231	45.050	-86.876	1.00	9.28	N
25	ATOM	2829	CA	ALA	183B	-64.929	45.699	-86.943	1.00	8.00	C
	ATOM	2830	CB	ALA	183B	-64.436	46.029	-85.539	1.00	8.10	C
	ATOM	2831	C	ALA	183B	-63.939	44.774	-87.649	1.00	6.64	C
	ATOM	2832	O	ALA	183B	-63.239	45.185	-88.572	1.00	5.70	O
	ATOM	2833	N	MET	184B	-63.900	43.518	-87.217	1.00	6.48	N
30	ATOM	2834	CA	MET	184B	-62.998	42.540	-87.806	1.00	9.92	C
	ATOM	2835	CB	MET	184B	-63.043	41.236	-87.009	1.00	12.68	C
	ATOM	2836	CG	MET	184B	-62.477	41.368	-85.604	1.00	14.45	C
	ATOM	2837	SD	MET	184B	-62.275	39.781	-84.796	1.00	16.52	S
	ATOM	2838	CE	MET	184B	-63.622	39.811	-83.645	1.00	16.57	C
35	ATOM	2839	C	MET	184B	-63.291	42.258	-89.279	1.00	10.76	C
	ATOM	2840	O	MET	184B	-62.372	42.120	-90.082	1.00	9.93	O
	ATOM	2841	N	GLN	185B	-64.567	42.169	-89.638	1.00	11.24	N
	ATOM	2842	CA	GLN	185B	-64.916	41.902	-91.027	1.00	13.68	C
	ATOM	2843	CB	GLN	185B	-66.420	41.643	-91.157	1.00	17.05	C
40	ATOM	2844	CG	GLN	185B	-66.911	40.452	-90.349	1.00	24.37	C
	ATOM	2845	CD	GLN	185B	-68.421	40.341	-90.330	1.00	28.17	C
	ATOM	2846	OE1	GLN	185B	-69.120	41.299	-89.998	1.00	30.13	O
	ATOM	2847	NE2	GLN	185B	-68.933	39.166	-90.678	1.00	30.64	N
	ATOM	2848	C	GLN	185B	-64.499	43.071	-91.918	1.00	11.65	C
45	ATOM	2849	O	GLN	185B	-64.149	42.881	-93.080	1.00	11.10	O
	ATOM	2850	N	LYS	186B	-64.522	44.282	-91.370	1.00	11.72	N
	ATOM	2851	CA	LYS	186B	-64.143	45.456	-92.146	1.00	10.73	C
	ATOM	2852	CB	LYS	186B	-64.859	46.702	-91.606	1.00	10.52	C
	ATOM	2853	CG	LYS	186B	-66.388	46.574	-91.661	1.00	11.83	C
50	ATOM	2854	CD	LYS	186B	-67.120	47.732	-91.001	1.00	13.74	C
	ATOM	2855	CE	LYS	186B	-66.983	49.016	-91.796	1.00	15.73	C
	ATOM	2856	NZ	LYS	186B	-68.014	50.019	-91.394	1.00	15.02	N
	ATOM	2857	C	LYS	186B	-62.638	45.676	-92.153	1.00	12.11	C
	ATOM	2858	O	LYS	186B	-62.060	45.974	-93.197	1.00	9.46	O
55	ATOM	2859	N	LEU	187B	-62.005	45.514	-90.992	1.00	12.40	N
	ATOM	2860	CA	LEU	187B	-60.562	45.715	-90.875	1.00	15.17	C
	ATOM	2861	CB	LEU	187B	-60.158	45.798	-89.400	1.00	13.66	C
	ATOM	2862	CG	LEU	187B	-58.670	45.952	-89.071	1.00	11.40	C
	ATOM	2863	CD1	LEU	187B	-58.124	47.248	-89.657	1.00	8.92	C
60	ATOM	2864	CD2	LEU	187B	-58.488	45.937	-87.560	1.00	9.50	C

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	ATOM	2865	C	LEU	187B	-59.741	44.627	-91.558	1.00	17.19	C
	ATOM	2866	O	LEU	187B	-58.842	44.923	-92.341	1.00	18.70	O
	ATOM	2867	N	HIS	188B	-60.052	43.371	-91.258	1.00	20.36	N
	ATOM	2868	CA	HIS	188B	-59.324	42.245	-91.834	1.00	22.73	C
5	ATOM	2869	CB	HIS	188B	-59.772	40.942	-91.169	1.00	20.87	C
	ATOM	2870	CG	HIS	188B	-59.606	40.934	-89.681	1.00	17.24	C
	ATOM	2871	CD2	HIS	188B	-59.933	40.004	-88.753	1.00	15.71	C
	ATOM	2872	ND1	HIS	188B	-59.049	41.987	-88.988	1.00	15.20	N
	ATOM	2873	CE1	HIS	188B	-59.040	41.706	-87.697	1.00	13.57	C
	ATOM	2874	NE2	HIS	188B	-59.571	40.509	-87.528	1.00	15.21	N
10	ATOM	2875	C	HIS	188B	-59.483	42.124	-93.349	1.00	26.46	C
	ATOM	2876	O	HIS	188B	-58.675	41.473	-94.011	1.00	25.55	O
	ATOM	2877	N	SER	189B	-60.521	42.747	-93.899	1.00	30.08	N
	ATOM	2878	CA	SER	189B	-60.756	42.685	-95.337	1.00	34.21	C
	ATOM	2879	CB	SER	189B	-62.258	42.706	-95.637	1.00	34.81	C
15	ATOM	2880	OG	SER	189B	-62.820	43.976	-95.354	1.00	35.57	O
	ATOM	2881	C	SER	189B	-60.087	43.841	-96.072	1.00	36.23	C
	ATOM	2882	O	SER	189B	-60.085	43.881	-97.299	1.00	36.65	O
	ATOM	2883	N	GLN	190B	-59.525	44.782	-95.320	1.00	38.28	N
	ATOM	2884	CA	GLN	190B	-58.861	45.936	-95.916	1.00	40.77	C
20	ATOM	2885	CB	GLN	190B	-58.387	46.897	-94.826	1.00	40.01	C
	ATOM	2886	CG	GLN	190B	-59.519	47.554	-94.066	1.00	40.20	C
	ATOM	2887	CD	GLN	190B	-60.461	48.302	-94.983	1.00	40.02	C
	ATOM	2888	OE1	GLN	190B	-60.051	49.217	-95.694	1.00	40.21	O
	ATOM	2889	NE2	GLN	190B	-61.732	47.913	-94.976	1.00	39.81	N
25	ATOM	2890	C	GLN	190B	-57.682	45.536	-96.792	1.00	42.89	C
	ATOM	2891	O	GLN	190B	-57.800	45.476	-98.015	1.00	43.99	O
	ATOM	2892	N	LYS	191B	-56.541	45.268	-96.170	1.00	44.78	N
	ATOM	2893	CA	LYS	191B	-55.359	44.881	-96.924	1.00	47.01	C
	ATOM	2894	CB	LYS	191B	-54.124	44.905	-96.017	1.00	46.09	C
30	ATOM	2895	CG	LYS	191B	-52.805	44.987	-96.771	1.00	44.63	C
	ATOM	2896	CD	LYS	191B	-52.739	46.261	-97.605	1.00	43.97	C
	ATOM	2897	CE	LYS	191B	-51.475	46.315	-98.446	1.00	43.98	C
	ATOM	2898	NZ	LYS	191B	-51.437	47.531	-99.304	1.00	43.71	N
	ATOM	2899	C	LYS	191B	-55.572	43.481	-97.497	1.00	49.43	C
35	ATOM	2900	O	LYS	191B	-56.295	42.669	-96.916	1.00	49.51	O
	ATOM	2901	N	ALA	192B	-54.950	43.209	-98.642	1.00	51.33	N
	ATOM	2902	CA	ALA	192B	-55.065	41.908	-99.299	1.00	52.55	C
	ATOM	2903	CB	ALA	192B	-54.435	40.825	-98.425	1.00	52.62	C
	ATOM	2904	C	ALA	192B	-56.520	41.553	-99.607	1.00	53.27	C
40	ATOM	2905	O	ALA	192B	-57.228	41.007	-98.759	1.00	53.50	O
	ATOM	2906	N	GLN	193B	-56.960	41.860	-100.826	1.00	53.92	N
	ATOM	2907	CA	GLN	193B	-58.332	41.578	-101.238	1.00	53.65	C
	ATOM	2908	CB	GLN	193B	-59.161	42.864	-101.212	1.00	52.88	C
	ATOM	2909	CG	GLN	193B	-58.997	43.674	-99.942	1.00	51.74	C
45	ATOM	2910	CD	GLN	193B	-59.889	44.898	-99.907	1.00	50.95	C
	ATOM	2911	OE1	GLN	193B	-61.109	44.786	-99.801	1.00	50.27	O
	ATOM	2912	NE2	GLN	193B	-59.282	46.076	-99.998	1.00	49.83	N
	ATOM	2913	C	GLN	193B	-58.382	40.972	-102.638	1.00	54.08	C
	ATOM	2914	O	GLN	193B	-58.777	39.793	-102.754	1.00	54.47	O
50	ATOM	2915	OXT	GLN	193B	-58.025	41.685	-103.600	1.00	54.16	O
	TER	2916		GLN	193B						
	ATOM	2917	OH2	WAT	500	-28.874	65.919	-68.085	1.00	2.43	O
	ATOM	2918	OH2	WAT	501	-67.611	53.748	-76.569	1.00	1.89	O
	ATOM	2919	OH2	WAT	502	-44.516	39.409	-89.610	1.00	5.41	O
55	ATOM	2920	OH2	WAT	503	-48.292	87.130	-65.406	1.00	2.69	O
	ATOM	2921	OH2	WAT	504	-43.342	77.146	-82.925	1.00	4.45	O
	ATOM	2922	OH2	WAT	505	-43.627	42.337	-68.612	1.00	2.91	O
	ATOM	2923	OH2	WAT	506	-41.774	50.201	-76.727	1.00	22.42	O
	ATOM	2924	OH2	WAT	507	-39.341	47.715	-74.095	1.00	2.10	O
60	ATOM	2925	OH2	WAT	508	-49.298	58.247	-58.763	1.00	4.60	O

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	ATOM	2926	OH2	WAT	509	-32.529	64.482	-64.542	1.00	1.00	O
	ATOM	2927	OH2	WAT	510	-33.551	64.045	-55.799	1.00	11.81	O
	ATOM	2928	OH2	WAT	511	-47.329	47.098	-78.494	1.00	23.19	O
	ATOM	2929	OH2	WAT	512	-24.429	74.395	-52.634	1.00	1.00	O
5	ATOM	2930	OH2	WAT	513	-29.444	83.554	-63.396	1.00	19.77	O
	ATOM	2931	OH2	WAT	514	-40.652	73.262	-84.245	1.00	4.33	O
	ATOM	2932	OH2	WAT	515	-48.873	65.717	-78.845	1.00	12.28	O
	ATOM	2933	OH2	WAT	516	-48.891	47.682	-89.653	1.00	5.80	O
	ATOM	2934	OH2	WAT	517	-34.143	71.695	-62.997	1.00	1.00	O
10	ATOM	2935	OH2	WAT	518	-45.245	90.255	-70.494	1.00	7.19	O
	ATOM	2936	OH2	WAT	519	-53.471	52.409	-58.930	1.00	7.74	O
	ATOM	2937	OH2	WAT	520	-55.905	73.433	-67.688	1.00	7.34	O
	ATOM	2938	OH2	WAT	521	-27.634	68.040	-51.809	1.00	17.79	O
	ATOM	2939	OH2	WAT	522	-66.200	70.718	-71.974	1.00	24.54	O
15	ATOM	2940	OH2	WAT	523	-40.178	52.478	-86.168	1.00	9.81	O
	ATOM	2941	OH2	WAT	524	-73.078	57.374	-86.967	1.00	21.70	O
	ATOM	2942	OH2	WAT	525	-37.382	52.796	-96.658	1.00	4.47	O
	ATOM	2943	OH2	WAT	526	-57.361	86.830	-65.333	1.00	3.20	O
	ATOM	2944	OH2	WAT	527	-26.448	72.367	-73.520	1.00	9.73	O
20	ATOM	2945	OH2	WAT	528	-26.998	80.928	-80.281	1.00	11.30	O
	ATOM	2946	OH2	WAT	529	-53.022	40.505	-79.031	1.00	5.42	O
	ATOM	2947	OH2	WAT	530	-36.785	90.413	-73.987	1.00	7.07	O
	ATOM	2948	OH2	WAT	531	-23.314	72.083	-51.179	1.00	6.51	O
	ATOM	2949	OH2	WAT	532	-60.473	58.993	-85.958	1.00	5.73	O
25	ATOM	2950	OH2	WAT	533	-41.935	58.655	-67.926	1.00	2.01	O
	ATOM	2951	OH2	WAT	534	-66.035	45.633	-75.837	1.00	8.61	O
	ATOM	2952	OH2	WAT	535	-48.936	69.151	-55.259	1.00	7.03	O
	ATOM	2953	OH2	WAT	536	-19.908	62.629	-62.163	1.00	4.52	O
	ATOM	2954	OH2	WAT	537	-41.558	47.570	-89.150	1.00	1.70	O
30	ATOM	2955	OH2	WAT	538	-48.711	80.727	-54.640	1.00	5.68	O
	ATOM	2956	OH2	WAT	539	-45.171	58.830	-61.103	1.00	4.30	O
	ATOM	2957	OH2	WAT	540	-42.233	65.934	-78.401	1.00	6.74	O
	ATOM	2958	OH2	WAT	541	-49.218	33.925	-76.362	1.00	23.24	O
	ATOM	2959	OH2	WAT	542	-29.959	63.826	-42.218	1.00	31.93	O
35	ATOM	2960	OH2	WAT	543	-33.616	74.094	-52.418	1.00	5.03	O
	ATOM	2961	OH2	WAT	544	-39.732	65.915	-68.894	1.00	5.31	O
	ATOM	2962	OH2	WAT	545	-42.575	62.172	-76.510	1.00	12.04	O
	ATOM	2963	OH2	WAT	546	-55.856	71.663	-75.992	1.00	31.22	O
	ATOM	2964	OH2	WAT	547	-46.227	57.928	-58.792	1.00	13.63	O
40	ATOM	2965	OH2	WAT	548	-32.073	39.106	-77.430	1.00	22.86	O
	ATOM	2966	OH2	WAT	549	-52.543	66.358	-76.164	1.00	4.12	O
	ATOM	2967	OH2	WAT	550	-49.297	65.045	-63.388	1.00	8.86	O
	ATOM	2968	OH2	WAT	551	-25.592	69.757	-73.599	1.00	16.87	O
	ATOM	2969	OH2	WAT	552	-58.804	65.116	-72.566	1.00	8.38	O
45	ATOM	2970	OH2	WAT	553	-26.130	74.821	-60.958	1.00	18.71	O
	ATOM	2971	OH2	WAT	554	-29.161	72.072	-47.281	1.00	15.10	O
	ATOM	2972	OH2	WAT	555	-27.107	71.690	-62.070	1.00	5.03	O
	ATOM	2973	OH2	WAT	556	-46.091	58.971	-63.882	1.00	5.87	O
	ATOM	2974	OH2	WAT	557	-33.487	55.967	-67.951	1.00	32.89	O
50	ATOM	2975	OH2	WAT	558	-52.213	63.538	-80.303	1.00	8.67	O
	ATOM	2976	OH2	WAT	559	-49.589	50.293	-58.619	1.00	7.20	O
	ATOM	2977	OH2	WAT	560	-40.917	57.601	-76.490	1.00	6.62	O
	ATOM	2978	OH2	WAT	561	-51.141	69.395	-63.223	1.00	4.76	O
	ATOM	2979	OH2	WAT	562	-18.297	83.463	-60.479	1.00	14.42	O
55	ATOM	2980	OH2	WAT	563	-54.682	61.774	-86.468	1.00	3.38	O
	ATOM	2981	OH2	WAT	564	-45.292	57.088	-65.407	1.00	3.47	O
	ATOM	2982	OH2	WAT	565	-54.396	69.950	-60.834	1.00	22.96	O
	ATOM	2983	OH2	WAT	566	-51.778	67.897	-80.011	1.00	29.72	O
	ATOM	2984	OH2	WAT	567	-31.724	58.107	-70.898	1.00	44.86	O
60	ATOM	2985	OH2	WAT	568	-37.649	74.884	-81.754	1.00	5.63	O
	ATOM	2986	OH2	WAT	569	-42.920	69.003	-83.056	1.00	5.50	O

	ATOM	2987	OH2	WAT	570	-56.313	54.666	-59.128	1.00	5.05	O
	ATOM	2988	OH2	WAT	571	-70.993	49.320	-84.273	1.00	19.64	O
	ATOM	2989	OH2	WAT	572	-40.627	89.783	-66.290	1.00	5.78	O
	ATOM	2990	OH2	WAT	573	-25.584	70.333	-66.683	1.00	8.26	O
5	ATOM	2991	OH2	WAT	574	-49.454	90.282	-53.536	1.00	23.08	O
	ATOM	2992	OH2	WAT	575	-39.180	56.595	-60.721	1.00	18.76	O
	ATOM	2993	OH2	WAT	576	-49.046	69.005	-61.766	1.00	3.32	O
	ATOM	2994	OH2	WAT	577	-50.548	88.363	-66.826	1.00	10.15	O
	ATOM	2995	OH2	WAT	578	-42.108	39.729	-90.878	1.00	5.77	O
	ATOM	2996	OH2	WAT	579	-49.637	81.204	-41.199	1.00	22.48	O
10	ATOM	2997	OH2	WAT	580	-45.803	22.824	-67.885	1.00	24.82	O
	ATOM	2998	OH2	WAT	581	-49.850	64.475	-80.842	1.00	16.19	O
	ATOM	2999	OH2	WAT	582	-43.697	58.079	-85.164	1.00	22.41	O
	ATOM	3000	OH2	WAT	583	-47.304	46.115	-58.797	1.00	23.89	O
	ATOM	3001	OH2	WAT	584	-82.717	38.969	-74.075	1.00	34.74	O
15	ATOM	3002	OH2	WAT	585	-49.715	89.341	-59.296	1.00	12.64	O
	ATOM	3003	OH2	WAT	586	-32.700	67.985	-53.140	1.00	4.17	O
	ATOM	3004	OH2	WAT	587	-45.974	58.694	-82.154	1.00	30.94	O
	ATOM	3005	OH2	WAT	588	-55.809	61.356	-93.345	1.00	13.49	O
	ATOM	3006	OH2	WAT	589	-20.650	75.264	-59.362	1.00	16.77	O
20	ATOM	3007	OH2	WAT	590	-66.548	52.138	-90.078	1.00	14.14	O
	ATOM	3008	OH2	WAT	591	-54.925	55.459	-95.849	1.00	7.81	O
	ATOM	3009	OH2	WAT	592	-68.779	49.738	-71.373	1.00	10.28	O
	ATOM	3010	OH2	WAT	593	-69.902	55.974	-97.961	1.00	27.85	O
	ATOM	3011	OH2	WAT	594	-21.182	61.829	-57.953	1.00	23.95	O
25	ATOM	3012	OH2	WAT	595	-83.212	39.144	-79.545	1.00	29.82	O
	ATOM	3013	OH2	WAT	596	-43.324	42.557	-94.894	1.00	24.02	O
	ATOM	3014	OH2	WAT	597	-34.856	104.038	-57.220	1.00	28.10	O
	ATOM	3015	OH2	WAT	598	-43.277	35.521	-82.872	1.00	16.35	O
	ATOM	3016	OH2	WAT	599	-47.665	40.407	-79.317	1.00	24.22	O
30	ATOM	3017	OH2	WAT	600	-41.789	57.503	-91.480	1.00	18.83	O
	ATOM	3018	OH2	WAT	601	-48.512	59.761	-91.146	1.00	20.86	O
	ATOM	3019	OH2	WAT	602	-26.123	59.056	-60.070	1.00	11.73	O
	ATOM	3020	OH2	WAT	603	-32.363	48.474	-81.838	1.00	13.98	O
	ATOM	3021	OH2	WAT	604	-26.840	66.220	-42.498	1.00	25.94	O
35	ATOM	3022	OH2	WAT	605	-38.264	43.071	-76.420	1.00	21.75	O
	ATOM	3023	OH2	WAT	606	-50.003	38.287	-79.803	1.00	42.89	O
	ATOM	3024	OH2	WAT	607	-62.839	40.276	-67.265	1.00	25.59	O
	ATOM	3025	OH2	WAT	608	-39.910	60.418	-53.586	1.00	20.71	O
	ATOM	3026	OH2	WAT	609	-63.719	50.729	-92.938	1.00	17.10	O
40	ATOM	3027	OH2	WAT	610	-22.127	71.675	-40.933	1.00	28.46	O
	ATOM	3028	OH2	WAT	611	-53.831	34.308	-88.924	1.00	22.50	O
	ATOM	3029	OH2	WAT	612	-54.287	83.976	-60.888	1.00	23.72	O
	ATOM	3030	OH2	WAT	613	-36.062	70.496	-69.379	1.00	21.98	O
	ATOM	3031	OH2	WAT	614	-43.307	75.217	-46.355	1.00	6.28	O
45	ATOM	3032	OH2	WAT	615	-28.396	86.785	-52.663	1.00	24.20	O
	ATOM	3033	OH2	WAT	616	-46.084	92.848	-53.878	1.00	20.15	O
	ATOM	3034	OH2	WAT	617	-60.814	38.073	-93.015	1.00	20.74	O
	ATOM	3035	OH2	WAT	618	-59.612	62.709	-68.596	1.00	36.20	O
	ATOM	3036	OH2	WAT	619	-51.248	83.905	-75.376	1.00	26.85	O
50	ATOM	3037	OH2	WAT	620	-44.099	51.578	-98.251	1.00	13.41	O
	ATOM	3038	OH2	WAT	621	-42.396	46.022	-94.960	1.00	8.24	O
	ATOM	3039	OH2	WAT	622	-66.845	48.492	-67.400	1.00	4.07	O
	ATOM	3040	OH2	WAT	623	-50.136	80.856	-49.996	1.00	26.60	O
	ATOM	3041	OH2	WAT	624	-53.952	95.314	-73.624	1.00	21.03	O
55	ATOM	3042	OH2	WAT	625	-73.662	40.830	-79.214	1.00	24.94	O
	ATOM	3043	OH2	WAT	626	-31.715	73.138	-79.112	1.00	23.50	O
	ATOM	3044	OH2	WAT	627	-36.603	65.701	-54.556	1.00	24.30	O
	ATOM	3045	OH2	WAT	628	-64.004	55.965	-69.412	1.00	6.63	O
	ATOM	3046	OH2	WAT	629	-62.949	68.690	-92.871	1.00	27.96	O
60	ATOM	3047	OH2	WAT	630	-49.783	35.513	-83.848	1.00	20.33	O

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	ATOM	3048	OH2	WAT	631	-63.598	78.422	-72.835	1.00	25.41	O
	ATOM	3049	OH2	WAT	632	-42.183	65.909	-51.544	1.00	21.38	O
	ATOM	3050	OH2	WAT	633	-41.945	58.527	-96.501	1.00	14.82	O
	ATOM	3051	OH2	WAT	634	-34.964	66.102	-42.146	1.00	15.56	O
5	ATOM	3052	OH2	WAT	635	-38.461	92.490	-65.765	1.00	23.14	O
	ATOM	3053	OH2	WAT	636	-67.825	40.637	-77.526	1.00	23.30	O
	ATOM	3054	OH2	WAT	637	-32.354	42.791	-83.903	1.00	14.98	O
	ATOM	3055	OH2	WAT	638	-44.596	56.901	-73.584	1.00	14.80	O
10	ATOM	3056	OH2	WAT	639	-48.876	71.976	-55.608	1.00	7.03	O
	ATOM	3057	OH2	WAT	640	-39.448	34.131	-88.064	1.00	11.99	O
	ATOM	3058	OH2	WAT	641	-39.917	93.162	-73.439	1.00	30.97	O
	ATOM	3059	OH2	WAT	642	-33.660	53.432	-92.069	1.00	31.39	O
	ATOM	3060	OH2	WAT	643	-41.670	52.447	-64.837	1.00	13.10	O
15	ATOM	3061	OH2	WAT	644	-29.324	62.498	-45.505	1.00	12.61	O
	ATOM	3062	OH2	WAT	645	-65.357	72.645	-60.451	1.00	25.51	O
	ATOM	3063	OH2	WAT	646	-28.651	88.071	-79.328	1.00	24.53	O
	ATOM	3064	OH2	WAT	647	-47.145	51.248	-72.486	1.00	9.60	O
	ATOM	3065	OH2	WAT	648	-35.866	56.253	-67.662	1.00	21.12	O
20	ATOM	3066	OH2	WAT	649	-49.423	84.893	-80.250	1.00	27.48	O
	ATOM	3067	OH2	WAT	650	-51.578	72.074	-76.054	1.00	20.26	O
	ATOM	3068	OH2	WAT	651	-36.003	66.896	-57.822	1.00	11.00	O
	ATOM	3069	OH2	WAT	652	-27.746	69.561	-68.222	1.00	6.61	O
	ATOM	3070	OH2	WAT	653	-35.305	62.489	-53.683	1.00	11.33	O
25	ATOM	3071	OH2	WAT	654	-37.366	66.471	-69.989	1.00	35.01	O
	ATOM	3072	OH2	WAT	655	-70.670	36.212	-82.244	1.00	23.57	O
	ATOM	3073	OH2	WAT	656	-44.047	57.339	-78.808	1.00	22.02	O
	ATOM	3074	OH2	WAT	657	-50.561	75.584	-76.121	1.00	20.22	O
	ATOM	3075	OH2	WAT	658	-15.309	58.222	-44.891	1.00	25.77	O
30	ATOM	3076	OH2	WAT	659	-46.225	62.145	-77.318	1.00	24.71	O
	ATOM	3077	OH2	WAT	660	-56.712	63.601	-62.294	1.00	14.58	O
	ATOM	3078	OH2	WAT	661	-40.389	73.960	-37.229	1.00	22.30	O
	ATOM	3079	OH2	WAT	662	-22.839	72.577	-63.807	1.00	18.09	O
	ATOM	3080	OH2	WAT	663	-57.975	65.550	-53.913	1.00	25.92	O
35	ATOM	3081	OH2	WAT	664	-71.192	41.260	-59.954	1.00	28.67	O
	ATOM	3082	OH2	WAT	665	-42.656	89.894	-77.859	1.00	24.66	O
	ATOM	3083	OH2	WAT	666	-45.603	54.220	-88.521	1.00	13.39	O
	ATOM	3084	OH2	WAT	667	-45.337	69.613	-50.352	1.00	16.39	O
	ATOM	3085	OH2	WAT	668	-34.536	37.234	-91.439	1.00	27.04	O
40	ATOM	3086	OH2	WAT	669	-45.247	60.927	-67.533	1.00	4.97	O
	ATOM	3087	OH2	WAT	670	-51.808	57.150	-89.675	1.00	14.11	O
	ATOM	3088	OH2	WAT	671	-16.807	66.275	-65.350	1.00	24.46	O
	ATOM	3089	OH2	WAT	672	-16.261	72.537	-57.458	1.00	15.98	O
	ATOM	3090	OH2	WAT	673	-42.178	70.005	-53.929	1.00	7.40	O
45	ATOM	3091	OH2	WAT	674	-52.244	94.415	-53.801	1.00	22.94	O
	ATOM	3092	OH2	WAT	675	-32.619	51.321	-76.288	1.00	37.06	O
	ATOM	3093	OH2	WAT	676	-45.403	48.572	-101.205	1.00	25.47	O
	ATOM	3094	OH2	WAT	677	-52.111	66.078	-62.228	1.00	24.34	O
	ATOM	3095	OH2	WAT	678	-61.750	69.333	-79.347	1.00	32.32	O
50	ATOM	3096	OH2	WAT	679	-27.179	66.842	-85.286	1.00	13.73	O
	ATOM	3097	OH2	WAT	680	-40.470	78.170	-36.171	1.00	25.27	O
	ATOM	3098	OH2	WAT	681	-47.219	34.290	-68.863	1.00	23.65	O
	ATOM	3099	OH2	WAT	682	-72.715	48.986	-51.050	1.00	32.18	O
	ATOM	3100	OH2	WAT	683	-26.828	77.224	-62.222	1.00	21.76	O
	ATOM	3101	OH2	WAT	684	-41.018	86.115	-80.528	1.00	20.41	O
55	ATOM	3102	OH2	WAT	685	-38.365	62.179	-65.591	1.00	6.86	O
	ATOM	3103	OH2	WAT	686	-41.541	62.923	-64.629	1.00	21.34	O
	ATOM	3104	OH2	WAT	687	-41.815	58.895	-65.055	1.00	11.86	O
	ATOM	3105	OH2	WAT	688	-40.894	62.440	-67.124	1.00	15.33	O
	ATOM	3106	OH2	WAT	689	-41.485	60.765	-69.666	1.00	17.52	O
60	ATOM	3107	OH2	WAT	690	-41.468	61.404	-72.328	1.00	12.37	O
	ATOM	3108	OH2	WAT	691	-40.102	66.180	-76.771	1.00	1.00	O

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	ATOM	3109	OH2	WAT	692	-39.806	62.467	-74.126	1.00	4.60	O
	ATOM	3110	OH2	WAT	693	-42.980	71.805	-83.551	1.00	2.52	O
	ATOM	3111	OH2	WAT	694	-41.580	65.253	-81.134	1.00	8.45	O
	ATOM	3112	OH2	WAT	695	-43.917	53.865	-83.738	1.00	21.44	O
5	ATOM	3113	OH2	WAT	696	-44.086	56.546	-82.755	1.00	15.41	O
	ATOM	3114	OH2	WAT	697	-42.412	51.507	-86.715	1.00	11.01	O
	ATOM	3115	OH2	WAT	698	-43.228	59.613	-77.468	1.00	27.31	O
	ATOM	3116	OH2	WAT	699	-42.404	59.741	-74.880	1.00	12.29	O
	ATOM	3117	OH2	WAT	700	-46.391	57.416	-80.153	1.00	16.42	O
10	ATOM	3118	OH2	WAT	701	-41.089	66.800	-61.597	1.00	18.81	O
	ATOM	3119	OH2	WAT	702	-37.974	67.845	-53.718	1.00	23.42	O
	ATOM	3120	OH2	WAT	703	-48.700	84.329	-51.893	1.00	14.05	O
	ATOM	3121	OH2	WAT	704	-46.271	87.086	-52.023	1.00	13.65	O
	ATOM	3122	OH2	WAT	705	-46.661	78.833	-50.542	1.00	14.53	O
15	ATOM	3123	OH2	WAT	706	-42.337	83.119	-49.717	1.00	11.09	O
	ATOM	3124	OH2	WAT	707	-50.418	82.903	-55.526	1.00	21.86	O
	ATOM	3125	OH2	WAT	708	-29.531	84.820	-57.540	1.00	19.33	O
	ATOM	3126	OH2	WAT	709	-30.116	87.453	-58.115	1.00	18.36	O
	ATOM	3127	OH2	WAT	710	-30.542	89.329	-60.666	1.00	19.84	O
20	ATOM	3128	OH2	WAT	711	-34.996	90.611	-68.847	1.00	8.05	O
	ATOM	3129	OH2	WAT	712	-54.120	66.823	-79.803	1.00	22.27	O
	ATOM	3130	OH2	WAT	713	-58.324	69.413	-77.071	1.00	19.75	O
	ATOM	3131	OH2	WAT	714	-48.943	62.144	-77.461	1.00	20.84	O
	ATOM	3132	OH2	WAT	715	-45.323	52.226	-71.160	1.00	16.49	O
25	ATOM	3133	OH2	WAT	716	-51.680	50.518	-60.235	1.00	7.07	O
	ATOM	3134	OH2	WAT	717	-46.126	52.624	-61.659	1.00	6.92	O
	ATOM	3135	OH2	WAT	718	-47.043	46.373	-63.059	1.00	20.47	O
	ATOM	3136	OH2	WAT	719	-59.718	67.072	-74.272	1.00	4.82	O
	ATOM	3137	OH2	WAT	720	-57.003	50.228	-59.428	1.00	9.27	O
30	ATOM	3138	OH2	WAT	721	-48.843	41.749	-61.131	1.00	8.47	O
	ATOM	3139	OH2	WAT	722	-43.235	57.074	-61.340	1.00	8.81	O
	ATOM	3140	OH2	WAT	723	-44.806	55.424	-58.022	1.00	18.53	O
	ATOM	3141	OH2	WAT	724	-43.084	61.057	-65.565	1.00	17.04	O
	ATOM	3142	OH2	WAT	725	-49.577	60.623	-57.745	1.00	8.64	O
35	ATOM	3143	OH2	WAT	726	-51.915	63.046	-56.367	1.00	23.76	O
	ATOM	3144	OH2	WAT	727	-51.541	67.717	-65.763	1.00	9.04	O
	ATOM	3145	OH2	WAT	728	-53.752	73.837	-69.450	1.00	5.30	O
	ATOM	3146	OH2	WAT	729	-55.969	76.184	-66.529	1.00	9.36	O
	ATOM	3147	OH2	WAT	730	-57.091	70.929	-64.486	1.00	23.11	O
40	ATOM	3148	OH2	WAT	731	-52.101	73.157	-73.654	1.00	16.55	O
	ATOM	3149	OH2	WAT	732	-52.586	75.551	-73.345	1.00	17.36	O
	ATOM	3150	OH2	WAT	733	-69.750	54.881	-75.453	1.00	9.46	O
	ATOM	3151	OH2	WAT	734	-72.039	53.568	-75.688	1.00	15.24	O
	ATOM	3152	OH2	WAT	735	-68.329	60.662	-81.143	1.00	21.03	O
45	ATOM	3153	OH2	WAT	736	-65.631	63.386	-72.476	1.00	26.21	O
	ATOM	3154	OH2	WAT	737	-39.896	79.834	-82.680	1.00	7.19	O
	ATOM	3155	OH2	WAT	738	-39.427	83.914	-83.210	1.00	20.00	O
	ATOM	3156	OH2	WAT	739	-55.916	58.914	-58.342	1.00	5.21	O
	ATOM	3157	OH2	WAT	740	-62.322	49.487	-59.298	1.00	20.88	O
50	ATOM	3158	OH2	WAT	741	-63.468	49.112	-55.511	1.00	23.08	O
	ATOM	3159	OH2	WAT	742	-58.861	62.061	-61.797	1.00	18.02	O
	ATOM	3160	OH2	WAT	743	-58.819	65.975	-69.609	1.00	24.05	O
	ATOM	3161	OH2	WAT	744	-62.183	57.343	-87.126	1.00	7.54	O
	ATOM	3162	OH2	WAT	745	-70.010	53.666	-82.601	1.00	10.37	O
55	ATOM	3163	OH2	WAT	746	-69.877	56.234	-84.527	1.00	12.50	O
	ATOM	3164	OH2	WAT	747	-72.977	49.645	-78.601	1.00	22.57	O
	ATOM	3165	OH2	WAT	748	-69.175	41.397	-81.847	1.00	10.89	O
	ATOM	3166	OH2	WAT	749	-61.141	50.911	-92.129	1.00	10.91	O
	ATOM	3167	OH2	WAT	750	-65.371	55.486	-89.978	1.00	20.17	O
60	ATOM	3168	OH2	WAT	751	-44.575	94.915	-54.555	1.00	15.35	O
	ATOM	3169	OH2	WAT	752	-69.165	43.817	-77.156	1.00	4.47	O

FIGURE 9 - 52

	ATOM	3170	OH2	WAT	753	-55.982	38.887	-84.625	1.00	15.37	O
	ATOM	3171	OH2	WAT	754	-60.493	37.315	-81.707	1.00	12.92	O
	ATOM	3172	OH2	WAT	755	-43.223	49.900	-88.973	1.00	6.43	O
	ATOM	3173	OH2	WAT	756	-55.017	45.768	-92.019	1.00	27.73	O
5	ATOM	3174	OH2	WAT	757	-44.952	44.733	-95.044	1.00	16.18	O
	ATOM	3175	OH2	WAT	758	-43.718	43.045	-80.231	1.00	5.67	O
	ATOM	3176	OH2	WAT	759	-45.942	40.566	-81.418	1.00	14.60	O
	ATOM	3177	OH2	WAT	760	-45.034	41.477	-66.281	1.00	12.84	O
	ATOM	3178	OH2	WAT	761	-51.550	38.694	-61.458	1.00	19.53	O
10	ATOM	3179	OH2	WAT	762	-42.740	62.341	-84.444	1.00	21.85	O
	ATOM	3180	OH2	WAT	763	-56.049	52.138	-58.025	1.00	7.41	O
	ATOM	3181	OH2	WAT	764	-51.437	87.770	-57.432	1.00	7.14	O
	ATOM	3182	OH2	WAT	765	-50.894	84.776	-57.652	1.00	12.04	O
	ATOM	3183	OH2	WAT	766	-41.068	96.729	-56.294	1.00	13.19	O
15	ATOM	3184	OH2	WAT	767	-42.400	41.975	-92.549	1.00	11.88	O
	ATOM	3185	OH2	WAT	768	-38.040	41.495	-91.375	1.00	21.71	O
	ATOM	3186	OH2	WAT	769	-34.544	47.462	-88.689	1.00	21.51	O
	ATOM	3187	OH2	WAT	770	-38.510	46.508	-95.240	1.00	17.21	O
	ATOM	3188	OH2	WAT	771	-37.184	49.644	-97.342	1.00	17.56	O
20	ATOM	3189	OH2	WAT	772	2.523	3.474	-1.217	1.00	18.99	O
	ATOM	3190	OH2	WAT	773	0.570	-1.642	-3.049	1.00	20.90	O
	ATOM	3191	OH2	WAT	774	-3.064	-2.461	5.287	1.00	19.90	O
	ATOM	3192	OH2	WAT	775	1.091	0.254	-4.668	1.00	19.49	O
	ATOM	3193	OH2	WAT	776	7.918	-6.547	0.362	1.00	15.05	O
25	ATOM	3194	OH2	WAT	777	0.233	1.951	4.212	1.00	23.94	O
	ATOM	3195	OH2	WAT	778	3.961	-4.043	6.036	1.00	16.59	O
	ATOM	3196	OH2	WAT	779	2.100	-3.174	-8.422	1.00	14.79	O
	ATOM	3197	OH2	WAT	780	-2.941	0.835	-5.354	1.00	16.24	O
	ATOM	3198	OH2	WAT	781	-0.585	7.197	2.315	1.00	18.00	O
30	ATOM	3199	OH2	WAT	782	-2.156	0.639	-2.928	1.00	17.79	O
	ATOM	3200	OH2	WAT	783	-0.385	5.861	-0.531	1.00	22.14	O
	ATOM	3201	OH2	WAT	784	6.438	6.917	-5.961	1.00	24.62	O
	ATOM	3202	OH2	WAT	785	5.781	5.006	3.972	1.00	18.23	O
	ATOM	3203	OH2	WAT	786	1.129	0.573	-2.150	1.00	19.51	O
35	ATOM	3204	OH2	WAT	787	-3.936	1.052	5.332	1.00	17.79	O
	ATOM	3205	OH2	WAT	788	1.740	-3.827	-4.998	1.00	21.02	O
	ATOM	3206	OH2	WAT	789	-0.586	1.220	1.915	1.00	20.74	O
	ATOM	3207	OH2	WAT	790	1.555	3.322	8.616	1.00	13.94	O
	ATOM	3208	OH2	WAT	791	-1.139	3.378	-1.612	1.00	20.00	O
40	ATOM	3209	OH2	WAT	792	5.372	-1.790	-4.262	1.00	19.68	O
	ATOM	3210	OH2	WAT	793	0.478	-4.223	-2.400	1.00	17.40	O
	ATOM	3211	OH2	WAT	794	-1.552	-1.533	-1.565	1.00	21.79	O
	ATOM	3212	OH2	WAT	795	-2.043	-3.685	-0.805	1.00	16.20	O
	ATOM	3213	OH2	WAT	796	1.327	2.105	0.154	1.00	26.24	O
45	ATOM	3214	OH2	WAT	797	2.684	-1.188	6.167	1.00	21.75	O
	ATOM	3215	OH2	WAT	798	-2.634	-1.015	0.687	1.00	19.78	O
	ATOM	3216	OH2	WAT	799	-0.700	8.291	-1.151	1.00	20.38	O
	ATOM	3217	OH2	WAT	800	-0.849	-6.679	-0.139	1.00	19.36	O
	ATOM	3218	OH2	WAT	801	-4.841	-4.586	8.701	1.00	14.17	O
50	ATOM	3219	OH2	WAT	802	-2.730	-4.596	1.205	1.00	19.89	O
	ATOM	3220	OH2	WAT	803	-4.608	2.418	3.303	1.00	22.79	O
	ATOM	3221	OH2	WAT	804	1.625	6.372	4.374	1.00	17.35	O
	ATOM	3222	OH2	WAT	805	-2.704	-0.316	3.073	1.00	16.90	O
	ATOM	3223	OH2	WAT	806	-2.396	0.709	-7.936	1.00	17.87	O
55	ATOM	3224	OH2	WAT	807	-2.103	9.519	-4.937	1.00	16.83	O
	ATOM	3225	OH2	WAT	808	-2.471	3.266	0.657	1.00	17.50	O
	ATOM	3226	OH2	WAT	809	-2.753	6.947	-2.234	1.00	22.55	O
	ATOM	3227	OH2	WAT	810	-0.871	5.046	-5.874	1.00	17.46	O
	ATOM	3228	OH2	WAT	811	-0.400	-1.342	-9.477	1.00	19.07	O
60	ATOM	3229	OH2	WAT	812	5.057	-1.388	6.893	1.00	20.15	O
	TER	3230		WAT	812						

FIGURE 9 - 53

FIGURE 10

	SP_pth	1 --MTKLIVGLGNPGDNYFETKHNVGFPHIDOLAKRQNVTFTHDKIFODDEASFFINGPKH
	EF_pth	1 ---MKLIVGLGNPGSKYKEIGHNIGFATIDEAYRQNVSFNNSN-FEADIAAEFFGTEKV
5	SA_pth	1 ---MKCIVGLGNICKPEELTRHNIGFEWVDVYILEKQNFSLDKOK-FKCGAYTIERMNGDKV
	EC_pth	1 -MTKLIVGLGNPGAEYAATRENAGAWFVDLAERPLRREAKFEYTSRVTEGGEDV
	PA_pth	1 MTAFOLVGLGNPGPEYDQTRHNAGALFVERLAHAACQGVSLVAERKIFSLVCKFSHQGKDV
	HP_pth	1 ---MTLIVGLGNPTLRYAHTRHAGEDHIDSLSVSELDHSFTFSPKHNAFLCVY---KDF
10	SP_pth	59 YLMKPTTFMNESGKAVHALEIHYGRDIDDLIETYDDLDMSFGKIRLRAGSAGGHNGIKS
	EF_pth	57 EALKPLTFMNESGRSVGPLEIYEGVDEEDLTIVYDDLDLEEKIRLRAGSAGGHNGIKS
	SA_pth	57 IFEPMPTMMNLSGEAVAPIMDYVNVPEDLIVIYDDLDLEOGOVRLIRAGSAGGHNGIKS
	EC_pth	60 RLLVPTTFMNLSGKAVAAMASFERIMPDEILVAHDELDLPPGVAKFKLGGHHGGHNGLKD
	PA_pth	61 RLLIPTTMNMRSGOSVAALAGEFERIAPDAILVAHDELDLPPGVAVLKTCGGHHGGHNGLKD
15	HP_pth	54 IFLKPOLYMMNLSGESVLSAKNEYKT--KELLIVEDDDLPICGVHFKNGGGNGHNGLKS
	SP_pth	119 IIQEHGTC-VFNR#RIGIGRPKNGMSVHHVLSKFDRDDYIG-LQSVDKVDDSVNYLOE
	EF_pth	117 IIAHLGTV-VFPRE#RIGIGRPSKNDVHHVLSITFPKTHEELLLAVKKAADAALEYACEG
	SA_pth	117 IIKMLGTD-QFGRERIGCMRPTNGMIVPDYVLORFSNDEMVTMEKVIHAAARATEKEMET
20	EC_pth	120 IISKLGNNPNEHLRLIGIHPGDKNKVGMGVLCRDPVSEQKLTIDEAARCTEMWFTD
	PA_pth	121 IIIAQLGNCNSFHRLRIGIHPGHSSIISGYVLGRAPRSEQELIDPSIDEALGVIPEMLAG
	HP_pth	112 IDLLCSN--SYYRLRNGISRG--IGVHEHVLSKFHNEEPLKNAFEEHAKNAKKEETES
25	SP_pth	178 ENFEKTMOPRNG-----
	EF_pth	176 ETEVETMNENPNKG-----
	SA_pth	176 SRFDHVMNNEFMGEVK-----
	EC_pth	180 GLTKATINRLHAFKAQ-----
	PA_pth	181 DWTRAMQKLHSQKA-----
	HP_pth	167 EDENAMONRETLKKPLKIES

30

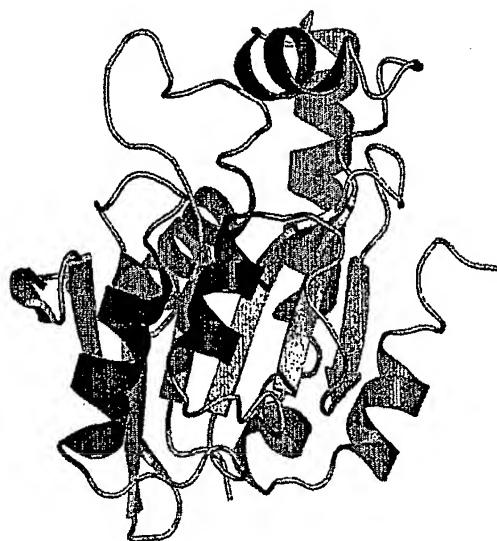
The species name abbreviations are as follows: SP, *Streptococcus pneumoniae*; EF, *Enterococcus faecalis*; EC, *Escherichia coli*; SA, *Staphylococcus aureus*; PA, *Pseudomonas aeruginosa*; HP, *Helicobacter pylori*.

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FIGURE 11



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FIGURE 12

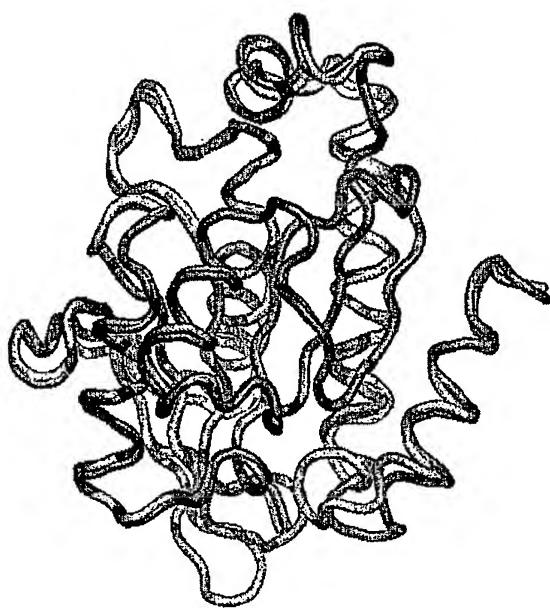
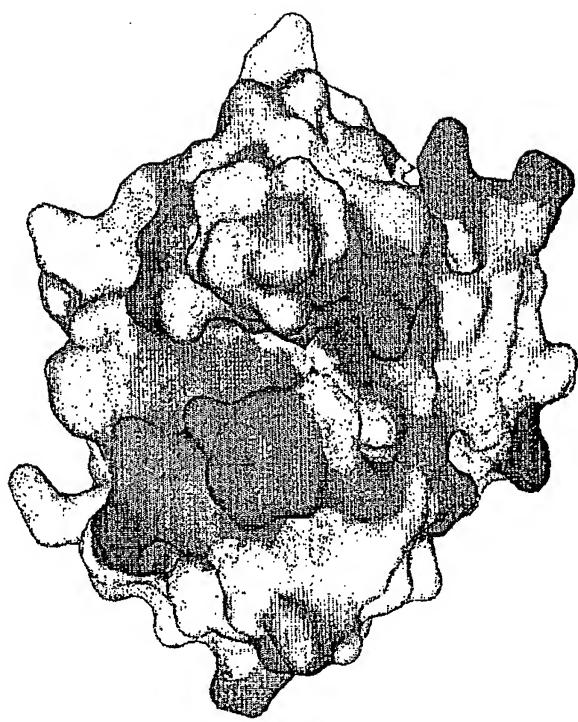


FIGURE 13



**FIGURE 14**



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